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**METHODS OF ANALYSIS**  
**A. O. A. C.**



OFFICIAL AND TENTATIVE  
METHODS OF ANALYSIS  
OF THE  
ASSOCIATION OF OFFICIAL  
AGRICULTURAL CHEMISTS

EDITORIAL BOARD

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W. F. REINDOLLAR, and MARIAN LAPP OTIS

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SIXTH EDITION, 1945

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## PREFACE TO SIXTH EDITION

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For this sixth edition the Revision Committee continues the custom of the Association of Official Agricultural Chemists to revise its *Official and Tentative Methods of Analysis* every five years. This revision includes the changes in methods and the additions adopted by the Association at the 59th annual meeting, October 1944.

For approximately 20 years a short title, "Methods of Analysis, A.O.A.C.," has been used by the Association for reference purposes, but to those actively engaged in laboratory work, it has become their "Book of Methods." The book is truly what this name implies, for in it are found scores of procedures for the analysis and examination of the products of or essential to agriculture, as well as other methods applicable to commodities only remotely, if at all, related to this fundamental industry of mankind.

The scope of the work and its important place in the progress of research and of the application of scientific methods to regulatory operations were clearly stated in 1920 by Dr. Harvey W. Wiley, the honorary president of the Association, in his introduction to the First Edition, part of which is quoted:

"The monumental work of the Association of Official Agricultural Chemists is not only well known in the profession in this country, but it is recognized in all countries as being the last word in agricultural chemical technique. The methods of determining the composition of agricultural products, as well as of all bodies related to agriculture, have been recognized also by the courts of this country.

"At the time of the organization of this body, agricultural methods of research, from the chemical point of view, were extremely chaotic. The progress of agricultural science which has marked its history in the last third of a century could not have been maintained amid these chaotic conditions. The methods adopted by the founders of this association for correcting this state of affairs have been shown by experience to be the best possible. I can say that the improvement in agricultural chemical technique has almost kept pace with the growth of the association.

"The gradual incorporation in the membership of the association of those scientific men engaged in the control of foods and drugs has widened the scope without altering the purpose of the original founders. Today we find a body of scientific workers in agriculture and related subjects numbering quite half a thousand, who, by their activities and collaboration, have contributed to the pages of this volume, directly or indirectly. The scientific knowledge of agriculture which has been verified and extended by this association now forms the foundation of all agricultural improvement."

The aim of the Association to keep abreast of the progress in technics and to meet the needs presented by the expanding problems of the enforcement official is reflected in this revision. A chapter in which no changes appear is exceptional indeed. Of greatest interest, however, are the new chapters on "Extraneous Materials in Foods and Drugs"; "Gelatin, Dessert Preparations, and Mixes"; and "Cosmetics," and the marked expansion of the methods for the determination of vitamins.

The Roman numerals for chapter identification are being replaced by Arabic numbers, and a decimal system of numbering the sections of each chapter is introduced in an attempt to make the book more convenient for everyday use by the worker at the bench. The sections of each chapter bear the chapter number to the left of the decimal and the consecutive section number to the right. In all cross references to other sections, whether in the same or in another chapter, the complete section number is employed.

The methods in this edition are those sponsored by the Association at the date of publication. Changes in the methods may be adopted at subsequent meetings, and the constitution provides that changes become effective thirty days after their publication in the first number of *The Journal* of the Association issued after any meeting. Purchasers, on request, will be supplied with reprints of reports on changes in the methods made each year in the interval between this and the next revision.

The publication of a compendium of this size is a task of no small magnitude for many workers. The appreciation of the Association and of those who find the book helpful and useful is to be extended to the Editorial Committee on *Methods of Analysis*, composed of Harry J. Fisher (*Chairman*), J. Walter Sale, L. E. Warren, W. H. Ross, W. F. Reindollar, and Marian Lapp Otis, and to the many referees, associate referees, collaborators, and members of various committees whose cooperation and untiring efforts were essential to this successful contribution to analytical science.

HENRY A. LEPPER  
*Secretary-Treasurer,  
Association of Official  
Agricultural Chemists*

Washington, D. C.  
September 15, 1945

*Abstract from*

## PREFACE TO FIRST EDITION

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"In presenting this revision of the official and tentative methods of analysis of the Association of Official Agricultural Chemists, it is appro-

priate to give a brief statement of the organization of the Association, its purpose, and the procedure by which the methods are adopted.

"Membership in the Association is institutional and includes the State Departments of Agriculture, the State Agricultural Colleges and Experiment Stations, the Federal Department of Agriculture, and the Federal, State, and City offices charged with the enforcement of food, feed, drug, fertilizer, insecticide and fungicide control laws.

"The Association was founded at Philadelphia, Pa., September 9, 1884, by the following representative agricultural chemists of that time, the organization being the result of a series of informal meetings held the immediately preceding years:

"Prof. H. W. Wiley, Chemist of the Department of Agriculture, Washington, D. C.

Mr. Clifford Richardson, Assistant Chemist of the Department of Agriculture, Washington, D. C.

Mr. Philip E. Chazal, State Chemist of South Carolina.

Dr. Chas. W. Dabney, Jr.,\* State Chemist of North Carolina.

Dr. W. J. Gascoyne, State Chemist of Virginia.

Dr. E. H. Jenkins, Connecticut Experiment Station.

Prof. John A. Myers, State Chemist of Mississippi.

Prof. H. C. White, State Chemist of Georgia.

Mr. C. DeGhequier, Secretary National Fertilizer Association.

Dr. Schumann, Dr. Lehmann, Mr. Gaines and others."

\* Only surviving charter member. (Dr. Dabney died June 15, 1945 )





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# DEFINITIONS OF TERMS AND EXPLANATORY NOTES

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(1) The term "water" means distilled water, except where otherwise specified, and except where the water does not mix with the determination.

(2) The term "alcohol" means 95% alcohol.

(3) The reagents listed below, unless otherwise specified, have the approximate strength stated and conform in purity with the Recommended Specifications for Analytical Reagent Chemicals of the American Chemical Society.

	<i>Specific gravity</i>
Sulfuric acid . . . . .	1.84
Hydrochloric acid . . . . .	1.184
Nitric acid . . . . .	1.42
Fuming nitric acid . . . . .	1.50
Acetic acid . . . . .	1.049 (25°)
Hydrobromic acid . . . . .	1.38
Ammonium hydroxide . . . . .	0.90
Phosphoric acid . . . . .	85% conc. by weight

(4) All other reagents and test solutions, unless otherwise described in the text, conform to the requirements of the American Chemical Society. When the anhydrous salt is intended, it is so stated; otherwise the salt referred to is the crystallized product.

(5) In the expressions (1+2), (5+4), etc., used in connection with the name of a reagent, the first numeral indicates the volume of the reagent used, and the second numeral indicates the volume of water. For example, hydrochloric acid (1+2) means a reagent prepared by mixing one volume of hydrochloric acid with two volumes of water. When one of the reagents is a solid, the expression means parts by weight, the first numeral representing the solid reagent and the second numeral the water.

(6) In making up solutions of definite percentage it is understood that *x* grams of substance is dissolved in water and made up to 100 ml. Although not theoretically correct, this procedure will not result in any appreciable error in any of the methods given in this book.

(7) For the sake of simplicity the abbreviations Cl and I instead of Cl<sub>2</sub> and I<sub>2</sub> are used for chlorine and iodine. Similar abbreviations have been used in other cases.

(8) All calculations are based on the table of international atomic weights, 44.1.

(9) Unless otherwise indicated all temperatures are expressed as degrees Centigrade.

(10) Directions for standardizing reagents are given in Chapter 43.

(11) To conserve space, the abbreviation "ca" is used for "approximately" or "about," and most of the articles have been eliminated.

# Official and Tentative Methods of Analysis

## OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

### 1. SOILS (1)—TENTATIVE

#### 1.1

##### DIRECTIONS FOR SAMPLING

(In view of the variability of soils, it seems impossible to devise an entirely satisfactory method for sampling. It is obvious that details of procedure should be determined by the purpose for which the sample is taken.)

Remove from surface all vegetable material not incorporated with soil. Take sufficient number of samples to insure a composite sample that will be representative of the tract sampled, to average depth of plowed soil, usually ca 7", and also take composite sample from each important and distinctly different soil stratum to depth of 40", using soil tube or auger. In using soil auger, enlarge first boring before boring below plowed depth and carefully clean out hole to prevent contamination of successive sub-strata while withdrawing samples. Obtain samples when soil is reasonably dry. Thoroly mix samples of each depth, sift thru a metal screen having circular openings of 2 mm diam., and dry in well ventilated, cool place. (When an analytical charge of finer screening is to be used, the proportion of the finer screening should be expressed as its percentage of the reserve 2 mm sample.)

To calculate percentage results obtained by analysis to pounds per given area of soil, determine weight of a given volume of soil as it lies in the field.

#### 1.2

##### PREPARATION OF SAMPLE

(a) Thru metal screen having circular openings of 2 mm diam. sift soil obtained under 1.1 and include material present as lumps that can be disintegrated by means of rubber-tipped pestle. Discard detritus, thoroly mix sifted material, and preserve in suitable stoppered container.

(b) When an analytical charge of finer rubber pestled screening, with or without grinding, is to be used, determine the proportion of finer screening in relation to the 2 mm reserve sample so that results of analyses can be expressed on preferred basis.

(c) If necessary for determination of total quantity of any constituent, *pulverize* more finely a sub-sample of (a).

#### 1.3

##### MOISTURE

Place 2 g of prepared sample, 1.2(a), in wide-mouthed weighing bottle and heat at 105° in electric oven for 5 hours. Report loss in weight as percentage based upon moisture-free weight of sample.

#### 1.4

##### LOSS ON IGNITION

(This method only approximates the organic matter content, especially for soils that contain much combined water.)

Ignite soil from 1.3 to full redness in Pt dish, stirring occasionally, until organic matter is destroyed. Cool in desiccator, weigh, and report percentage loss in weight as "loss on ignition." Utilize residue for  $\text{Na}_2\text{CO}_3$  fusion, 1.11.

### CARBONATE CARBON

#### 1.5

#### APPARATUS

*Quadruplicate shaking apparatus for evolution of carbonate carbon in soils of high or low carbon content.*—Apparatus (Figs. 1 and 2) consists of horizontal holder (H) 21" long,  $\frac{3}{4}$ " thick, and  $1\frac{1}{4}$ " wide, having properly spaced slots made to fit loosely the neck of a 300 ml Erlenmeyer flask taking a No. 6 rubber stopper. The holder

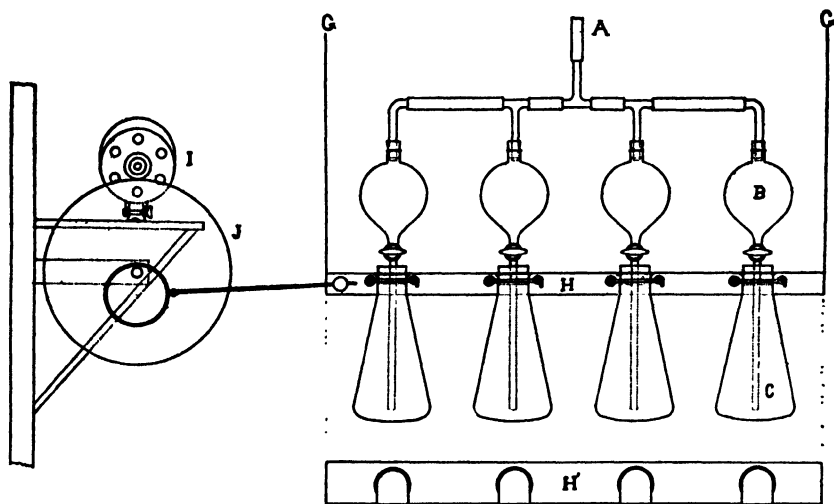


FIG. 1.—QUADRUPLICATE SHAKING APPARATUS FOR DETERMINATION OF CARBONATE CARBON IN SOILS

is suspended horizontally from a bar by means of brass strips  $1\frac{1}{4}$ " wide and 24" long. Air leading to A is purified by passing thru beaded tower 1"  $\times$  25", containing 10% NaOH, set into liter suction flask. Top of tower is provided with large N distillation bulb.

Driving wheel (J) is  $\frac{3}{4}$ " thick and 7" in diam. Eccentric attached to its face is  $\frac{3}{4}$ " thick and 2" in diam., and is grooved to permit free rotation of driving shaft, which is fastened to end of holder by means of a binding post. Power for agitation is supplied by motor (I), a sewing machine or small desk fan motor. If motor available has no rheostat, its speed can be easily controlled by battery of 4 lamps. Motor is hinged upright on the support so that pulley will rest upon edge of driving wheel. To reduce noise the pulley of motor is inserted into rubber stopper; or driving wheel may be made to carry a belt that is driven by pulley of small motor.

Absorption tower (D) is at least 25" high and 1" in diam. It contains alternating pockets of solid glass rods and small glass beads that rest upon an inverted test tube  $2\frac{1}{4}$ " long. The rubber connection on intake cock of tower is used to disconnect the glass tube that extends to rubber connection on safety bulb tube leading from flask C.

## DETERMINATION

## 1.6

*Volumetric Method (2)*

Pulverize sample to pass 60-mesh sieve. For soils low in carbonates use 10, 25, or 50 g charge in quadruplicate shaking device, 1.5.

Introduce charge into 300 ml evolution flask (C, Figs. 1 and 2), and aspirate 5 min. to free apparatus of atmospheric  $\text{CO}_2$ ; release suction, and introduce 10, 25, or 50 ml of 0.5 *N* NaOH soln into absorption tower. Apply a suction of 5" and introduce 60 ml of HCl (1+9) containing 5% of  $\text{SnCl}_2$  upon soil contained in Erlenmeyer flask, regulating intake of air by means of a screw clamp placed just beyond absorption tower. Agitate and aspirate 60 min. at rate of 3–4 bubbles per second. Then release suction and draw off absorbent soln into 500 ml flask, washing tower with a succession of fillings of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  to volume of 450 ml. Add 10 ml of neutral aqueous soln of  $\text{BaCl}_2$  (250 g of  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  per liter), make to volume, agitate, and allow to stand 4 hours. Titrate excess of hydroxide, using phenolphthalein indicator. With small-bore buret permitting split-drop readings to hundredths of a ml, use 0.5 *N* acid for titration; with burets of larger bore, use 0.1 or 0.25 *N* acid. Report result as percentage of  $\text{CO}_2$ .

## 1.7

*Gravimetric Method (3)*

Proceed as in 1.6, but in lieu of the NaOH absorbent and the absorption tower, Fig. 2, use an absorption tube filled with ascarite, preceded in train by tubes containing an  $\text{Ag}_2\text{SO}_4$  suspension in  $\text{H}_2\text{SO}_4$  (1+19),  $\text{H}_2\text{SO}_4$ , and anhydron, in order. Report increase in weight found for ascarite tube in percentage of  $\text{CO}_2$ .

NOTE.—Special consideration should be given to soils that have been treated with magnesite or dolomite and those known to be derived from the limited magnesite area, or from the glaciated region where transported dolomite may occur in considerable quantities. For such soils, agitate the HCl– $\text{SnCl}_2$ –soil suspension until  $\text{CO}_2$  evolution has subsided. Then apply heat to agitated suspension, with provision for condensation, until no  $\text{CO}_2$  evolution is indicated by the liquids in absorption train. Remove heat, discontinue agitation, and draw  $\text{CO}_2$ -free air thru apparatus 20 min. The absorption may be accomplished by either volumetric (1.6) or gravimetric (1.7) procedure.

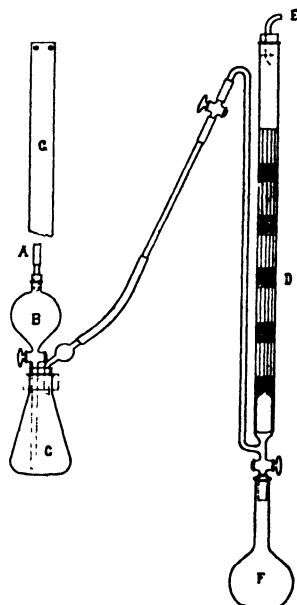


FIG. 2.—ABSORPTION TOWER

## ORGANIC CARBON (4)

## 1.8

## APPARATUS

(a) *Oxygen cylinder*.—With pressure-regulating valve.

(b) *Electric combustion furnace*.—With rheostat, and with  $\frac{1}{2} \times 24$ " fused silica tube containing a 4" loosely packed core of platinized asbestos. ( $\text{CuO}$  may be used if temp. of  $950^\circ$  is not exceeded.)



(c) *Purification and absorption train.*—Place 2 scrubber bottles containing 10% KOH soln, followed by Hg valve, between O supply and intake end of furnace. Provide an asbestos-filled Cu coil with handle as insulating plug on intake end of combustion tube; also use asbestos plug to insulate rubber stopper at outlet end of combustion tube. The outcoming current is dried and purified by an  $\text{H}_2\text{SO}_4$  scrubber, a tube containing 40-mesh granulated Zn, and a tube of  $\text{P}_2\text{O}_5$ , or other effective drying material, in order. Connect drying tube with Nesbit or similar absorption tube filled with alternate layers of glass wool and ascarite and protect against moisture and back pressure at its outlet by a Fisher bubble counter containing  $\text{H}_2\text{SO}_4$ .

## 1.9

## DETERMINATION

Bring furnace to  $950^\circ$ . Connect train and sweep out apparatus by adjusted flow of O. Weigh absorption tube against a counterpoise, replace it in train, and introduce well within heated zone an alundum boat containing 2 g charge of soil admixed with 2 g of finely divided CuO. Close intake and open Nesbit bulb. When no more gas passes thru absorption train, connect with suction, admit a flow of O, and aspirate 30 min. Close Nesbit tube, disconnect, and weigh against counterpoise. Correct total evolution of  $\text{CO}_2$  for carbonate  $\text{CO}_2$ , 1.6 or 1.7, and report as organic C, or  $\text{CO}_2$ . (There should be no carbonates in residue.)

## 1.10

## TOTAL NITROGEN

Digest 10 g of soil in a 500 ml Kjeldahl flask with 30–40 ml of  $\text{H}_2\text{SO}_4$  and ca 10 g of salt mixture composed of 10 parts of  $\text{K}_2\text{SO}_4$  or anhydrous  $\text{Na}_2\text{SO}_4$ , 1 part of  $\text{FeSO}_4$ , and  $\frac{1}{2}$  part of  $\text{CuSO}_4$ . Continue digestion until mixture is colorless or nearly so. After cooling, dilute contents of flask with  $\text{H}_2\text{O}$ , add excess of ca 45% NaOH soln; connect flask with condenser and distil 150 ml into standard acid, as directed under 2.24. (Distillation may be carried out in digestion flask, or, if preferred, soln may be transferred to an Armsby Cu pot.) Titrate excess of acid with 0.1 N or N/14 alkali, using methyl red or cochineal indicator, 2.22(h) and 2.22(i). Report as percentage of N.

## 1.11

## SODIUM CARBONATE FUSION (5)

In 30 ml Pt crucible, mix residue from 1.4 with 10 g of  $\text{Na}_2\text{CO}_3$ . Cover crucible and heat at low redness until fusion begins; increase heat to a clear, quiet fusion; then give full heat of Meker burner 20 min., with flame oblique. Cool the melt, place in 250 ml porcelain evaporating dish, add 100 ml of  $\text{H}_2\text{O}$ , and digest to disintegration on water bath. Cover dish, add 50 ml of HCl, digest 15 min., and wash cover. Evaporate to dryness and bake 2 hours at  $110^\circ$  (or substitute beaker for dish and 15 ml of  $\text{HClO}_4$  for HCl and evaporate to fumes, thereby obviating baking).

## 1.12

## SILICA

Take up the residue from 1.11 in HCl (1+9) (or dilute the  $\text{HClO}_4$  soln) and filter mixture so obtained. Wash with hot  $\text{H}_2\text{O}$  containing 5 ml of HCl per liter. Collect filtrate and washings in dish, preferably a casserole, and dehydrate on steam bath until the  $\text{SiO}_2$  assumes a crystalline appearance. Moisten with HCl and repeat dehydration 2 hours. Add 5 ml of HCl and 100 ml of hot  $\text{H}_2\text{O}$ , mix thoroly, filter, and wash with hot  $\text{H}_2\text{O}$  containing 5 ml of HCl per liter. Add residue to main portion of  $\text{SiO}_2$  obtained from first filtration. Make up combined filtrate and washings to 500 ml and use in subsequent determinations. (For soils of low Ca content, use the entire filtrate.) Place the two  $\text{SiO}_2$  residues with filters in a Pt crucible. Moisten

with saturated  $\text{NH}_4\text{NO}_3$  soln. Ignite with low heat at first to burn off filter paper and then with strong flame, preferably a blast lamp, to constant weight; cool in desiccator and weigh. Report as percentage of  $\text{SiO}_2$ .

### 1.13 OXIDES OF IRON, ALUMINUM, PHOSPHORUS, AND TITANIUM

To 250 ml aliquot of filtrate from  $\text{SiO}_2$ , 1.12, add 10 ml of  $\text{HCl}$  and few drops of methyl red indicator; heat to gentle boiling and add  $\text{NH}_4\text{OH}$  (1+1) until precipitate forms and the indicator in soln just changes to a distinct yellow. Boil not longer than 2 min. and filter rapidly. Wash precipitate 6–8 times with hot 2%  $\text{NH}_4\text{NO}_3$  soln. Return precipitate and filter to original beaker, add 10 ml of  $\text{HCl}$ , and macerate filter with policeman. Dilute with  $\text{H}_2\text{O}$ , heat to dissolve precipitate, dilute to ca 200 ml, and reprecipitate as directed above. Wash thoroly with hot  $\text{NH}_4\text{NO}_3$  soln until free of chlorides. Combine first and second filtrates and save for Ca and Mg determinations.

Place precipitate in Pt crucible, dry, and ignite over low flame of Bunsen burner until C has been oxidized. Heat to bright redness ca 10 min. Cool in desiccator and weigh in covered crucible as  $\text{Fe}_2\text{O}_3$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{P}_2\text{O}_5$ , and  $\text{TiO}_2$ .

### 1.14 CALCIUM

Concentrate combined filtrates and washings from 1.13 to ca 50 ml; make slightly alkaline with  $\text{NH}_4\text{OH}$  (1+1); and add, while still hot, saturated  $\text{NH}_4$  oxalate soln dropwise as long as any precipitate is produced, and then an excess sufficient to convert the Mg salts also to oxalate. Heat to boiling, allow to stand 3 hours or longer, decant clear soln thru filter, pour 15–20 ml of hot  $\text{H}_2\text{O}$  on precipitate, and again decant clear soln thru filter. Dissolve any precipitate remaining on filter by washing with hot  $\text{HCl}$  (1+9) into original beaker, wash 6 times with hot  $\text{H}_2\text{O}$ , and then reprecipitate, boiling hot, by adding  $\text{NH}_4\text{OH}$  and a little  $\text{NH}_4$  oxalate soln. Allow to stand as before and filter thru same filter. Wash free from chlorides with hot  $\text{H}_2\text{O}$ . Reserve filtrates and washings from both precipitations for determination of Mg, 1.16. Complete determination by one of following procedures and report as percentage of CaO:

(a) Ignite precipitate in a crucible either over an S-free blast or in electric oven at  $950^\circ$  to constant weight, cool in desiccator, and weigh CaO.

(b) Incinerate filter over low flame, mix ignited precipitate with finely pulverized and dried mixture of equal parts of  $(\text{NH}_4)_2\text{SO}_4$  and  $\text{NH}_4\text{Cl}$ , and drive off excess of sulfate by careful heating of upper portion of crucible. Complete ignition, cool in desiccator, and weigh the  $\text{CaSO}_4$  (6).

(c) Perforate apex of cone; wash Ca oxalate precipitate into beaker used for precipitation; and then wash filter with hot  $\text{H}_2\text{SO}_4$  (1+4) and titrate hot ( $85\text{--}90^\circ$ ) with 0.1 N  $\text{KMnO}_4$ .

### MAGNESIUM (7)

#### 1.15

#### REAGENT

*Phosphate soln.*—Dissolve 100 g of  $(\text{NH}_4)_2\text{HPO}_4$  in hot  $\text{H}_2\text{O}$ , dilute to 1 liter, and introduce 5 ml of  $\text{CHCl}_3$ .

#### 1.16

#### DETERMINATION

To combined filtrates and washings, 1.14, add 2 ml of 1 M citric acid, 100 ml of  $\text{NH}_4\text{OH}$ , and 50 ml of alcohol. Then add with constant stirring 25 ml of the phosphate soln and let stand 12–24 hours. Filter, wash twice with  $\text{NH}_4\text{OH}$  (1+9), and dissolve precipitate in  $\text{HNO}_3$  (1+4), washing soln into original beaker to volume

of 100–150 ml. To this soln add  $\frac{1}{10}$  volume of  $\text{NH}_4\text{OH}$  and 2 drops of the phosphate soln. Stir vigorously and allow to stand 3 hours or longer. Filter thru a Gooch crucible, wash with  $\text{NH}_4\text{OH}$  (1+9), moisten filter with saturated ammoniacal soln of  $\text{NH}_4\text{NO}_3$ , ignite, and weigh as  $\text{Mg}_2\text{P}_2\text{O}_7$ . Report as percentage of  $\text{MgO}$ . Correct weight of  $\text{Mg}_2\text{P}_2\text{O}_7$  for occluded  $\text{Mn}_2\text{P}_2\text{O}_7$  as directed in 37.74.

## 1.17

## MANGANESE

Treat 1 g of 100-mesh soil with 5 ml of  $\text{HF}$  and 5 ml of  $\text{H}_2\text{SO}_4$  (1+1); evaporate and heat gently to dryness. Repeat addition of  $\text{HF}$  until all silicates are decomposed. Dissolve in  $\text{H}_2\text{O}$ , add  $\text{HNO}_3$ , and evaporate to dryness. Again dissolve in  $\text{H}_2\text{O}$ , add 25 ml of  $\text{HNO}_3$  (1+2) and ca 0.5 g of Na bismuthate, and heat until permanganate color disappears. Proceed as directed under 37.76, beginning, "Add a few drops of a 10% soln of  $\text{NH}_4$  bisulfite or saturated Na bisulfite to clear soln." Report as percentage of  $\text{Mn}_2\text{O}_4$ .

## IODINE (8)

## 1.18

*Fusion Method*

Place in a clean 400 ml iron crucible 5 g of air-dried soil, ground to pass a 100-mesh sieve, 10 g of I-free  $\text{KOH}$  pellets, and 5 ml of  $\text{H}_2\text{O}$ , and stir with clean piece of No. 6 iron wire until most of pellets have dissolved. Place crucible in 4.5" Bunsen tripod and heat moderately with flame of burner, stirring contents of crucible rapidly until  $\text{H}_2\text{O}$  has been driven off and dry granular fused mass remains. Avoid heating crucible to redness after  $\text{H}_2\text{O}$  has been expelled. Cool crucible and add ca 50 ml of  $\text{H}_2\text{O}$  and allow to stand, with occasional stirring, until the fused mass has slaked to a sludge. Transfer contents of crucible to 500 ml beaker, police, and wash inside walls of crucible thoroly. Add small strip of litmus paper, ca 0.1 g of K bisulfite, and  $\text{HCl}$  (1+1), stirring until contents of beaker have acid reaction and distinct odor of  $\text{SO}_2$  can be detected. Add saturated  $\text{K}_2\text{CO}_3$  soln from a short-stemmed pipet, stirring until entire mass has an alkaline reaction. Pour precipitate of  $\text{SiO}_2$ ,  $\text{Fe}$  and  $\text{Al}$  hydroxides onto folded filter and wash thoroly by addition of ca 25 ml portions of hot  $\text{H}_2\text{O}$  at a time, allowing each portion to drain thru before adding another. (Volume of filtrate and washings should be ca 500 ml.) Transfer filtrate to porcelain dish and evaporate until a sludge of salts remains. (A small current of compressed air directed on surface of soln during heating on water bath will hasten evaporation.) Remove dish from water bath and add 50 ml of pure alcohol to the hot sludge of salts. Stir thoroly with a policeman until dish has attained room temp. (The salts assume a pasty consistency with much stirring while they are hot, and this condition facilitates soln of the  $\text{KI}$  in the alcohol.) Decant the alcoholic extract thru small folded filter into beaker, further extract residue with one 25 ml and one 10 ml portion of alcohol, and decant thru filter into beaker containing first alcoholic extract. Dissolve salts that adhere to filter paper and those in dish in hot  $\text{H}_2\text{O}$ , and evaporate soln to a sludge. Extract sludge with one 25 ml and two 10 ml portions of alcohol, as directed in treatment of first sludge of salts, and repeat process of extraction, using three 10 ml portions of alcohol. Combine alcoholic extracts and evaporate to dryness. Dissolve residue in small quantity of hot  $\text{H}_2\text{O}$ , rinse into 250 ml beaker, and evaporate to dryness. Dissolve residue in few drops of hot  $\text{H}_2\text{O}$  and a drop of saturated  $\text{K}_2\text{CO}_3$  soln, and add 10 ml of alcohol. Stir precipitated salts rapidly with glass rod ca 10 min., and decant alcoholic extract thru small filter into 150 ml beaker. Further stir residue, extract with two 5 ml portions of alcohol, decant thru filter into beaker, rinsing filter with a few ml of alcohol, and evaporate extract to dryness. Dissolve residue, consisting of ca 0.05 g, in few drops of hot  $\text{H}_2\text{O}$  and rinse

into 25 ml Pt or Sillimanite dish, evaporate to dryness, and dry at 100° 1 hour. Heat dish at ca 400° in electric furnace having pyrometer attachment until organic matter is burned or charred so that it will not give a turbid soln when H<sub>2</sub>O is added. If turbid soln is obtained, evaporate to dryness and burn again. After cooling dish, dissolve residue in few drops of H<sub>2</sub>O at room temp., filter soln, and wash into 30 ml separator. (Soln should be colorless, slightly alkaline, and have volume of ca 5 ml.) Make slightly acid with a few drops of H<sub>2</sub>SO<sub>4</sub> (1+1), add ca 0.01 g of pure K bisulfite to separator, stopper, and shake a few seconds to reduce any iodate to iodide. Remove stopper, and add 1 ml of pure CS<sub>2</sub> and ca 2 ml of 10% soln of pure I-free KNO<sub>3</sub> or NaNO<sub>3</sub>. Stopper, and shake separator vigorously ca 1 min. Place

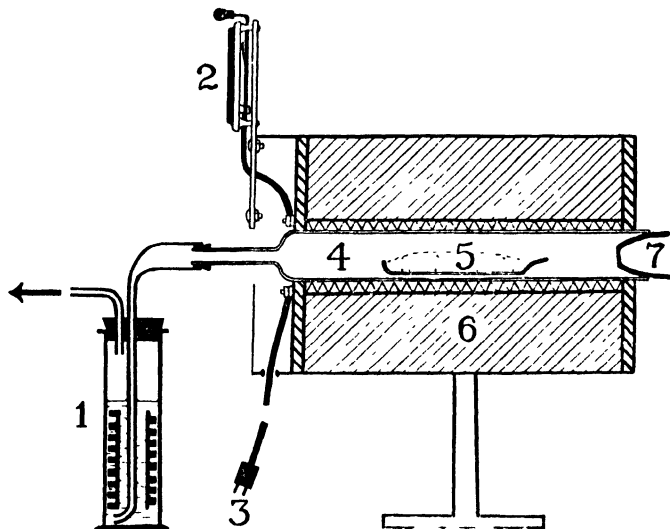


FIG. 3—FURNACE USED IN DETERMINATION OF IODINE IN SOILS

1, gas wash bottle; 2, rheostat; 3, power line, 220 volts; 4, quartz tube; 5, sample (in Sillimanite boat); 6, electric tube furnace; 7, stopper (alundum crucible).

separator in stand and allow the CS<sub>2</sub> containing the I to collect and settle ca 5 min. If the CS<sub>2</sub> is light pink in color, it contains all the I; if it is a deep pink, run it carefully into centrifuge tube and further extract soln in separator with 1 ml portions of CS<sub>2</sub> until last portion is only slightly pink. Combine CS<sub>2</sub> extracts and centrifuge. Place a portion of clear extract in micro cup of colorimeter and compare quickly with freshly prepared I standard having comparable depth of color. Report results in p.p.m.

#### 1.19 Volatilization Method by Heating Soil in an Electric Tube Furnace

Place in porcelain boat 25–100 g of soil ground to pass 2 mm sieve and insert boat in silica tube, Fig. 3. Use 2 wash bottles (Milligan), each containing 100 ml of 2.5% K<sub>2</sub>CO<sub>3</sub> soln. Connect first wash bottle with silica tube by means of a glass thimble made to fit a rubber gasket on small end of combustion tube. Connect wash bottles closely with rubber tubing. Attach the last wash bottle to suction pump regulated to draw vapors at a moderate rate into the wash bottles during time the soil is heated. (About 1 hour is required to attain maximum temp. of furnace, which is

maintained ca 2 hours.) Disconnect wash bottles, rinse soln into porcelain dish, and evaporate to dryness. Dissolve residue in few drops of hot  $\text{H}_2\text{O}$ , rinse into 150 ml beaker, and evaporate until ca 2 ml remains. Add to beaker 10 ml of pure alcohol, stir rapidly with glass rod ca 10 min., and decant extract thru small filter into 150 ml beaker. Extract residue with two 5 ml portions of alcohol and decant thru filter into beaker. Evaporate alcohol and wash residue into 25 ml Sillimanite dish; evaporate to dryness, dry at  $100^\circ$ , and heat at ca  $400^\circ$  for 10 min. in electric furnace having pyrometer attachment. Remove dish from furnace; cool, and dissolve residue in few drops of cold  $\text{H}_2\text{O}$ . Filter, and wash into 30 ml separator. Liberate, absorb, and determine I as directed in 1.18.

### SULFUR (9)

#### 1.20

#### PREPARATION OF SOLUTION

Weigh 5–10 g of the soil, 1.2(a), prepared to pass 0.5-mm. sieve, into 100 ml Ni crucible; add equal weight of anhydrous  $\text{Na}_2\text{CO}_3$ ; and mix well with stout Ni stirring rod of such length as to permit introduction into furnace to be used in fusion. Pipet carefully 4 ml of  $\text{H}_2\text{O}$  into each 10 g of soil; stir well to a stiff paste, adding any necessary  $\text{H}_2\text{O}$  dropwise. Immediately add successive portions of ca 1 g of S-free  $\text{Na}_2\text{O}_2$ , stirring well after each addition to obviate excessive frothing and overflow. Continue to add peroxide until mixture becomes dry and granular, and add, as a surface coating, enough to make total peroxide addition 25 g/10 g of soil. Place mixture in electric furnace; maintain temp. at  $400\text{--}500^\circ$  during first half hour, then raise rapidly to bright red heat (ca  $900^\circ$ ); and continue fusion at this temp. ca 10 min. Withdraw crucible from muffle, quickly manipulate so as to cause melt to spread out in thin sheet over interior of crucible, and cool rapidly by contact with some good conductor in cool atmosphere. Place chilled crucible sidewise in 600 ml beaker and cover with  $\text{H}_2\text{O}$ . Add ca 5 ml of alcohol to decompose the Na manganate. Cover beaker with watch-glass, place on cold hot plate, and apply heat. Boil briskly until melt is disintegrated (30 min.). When suspension has assumed a flesh-colored, flocculent appearance, with no glassy green lumps in interior of crucible, remove crucible and rod from beaker and wash any flaky particles back into beaker with a policeman, rinsing several times with hot  $\text{H}_2\text{O}$ . (If small glassy particles still cling to inside of crucible, disintegrate by boiling  $\text{H}_2\text{O}$  over hot plate or small flame and add to main portion.) Filter immediately by suction thru 9 cm Büchner funnel into liter beaker placed under bell jar. When no more liquid can be drawn thru filter, return residue, together with filter paper, to original beaker, washing any adhering particles carefully from funnel. Add ca 1 g of  $\text{Na}_2\text{CO}_3$ , macerate with the policeman, add 75–100 ml of  $\text{H}_2\text{O}$ , and bring to brisk boil while stirring vigorously. Again filter thru Büchner funnel, using suction until nearly dry, and wash with 20 ml portions of hot  $\text{H}_2\text{O}$  to total volume of 500 or 700 ml for the 5 or 10 g charge, respectively.

#### 1.21

#### DETERMINATION

Add from buret slowly and with stirring, sufficient  $\text{HCl}$  to neutralize the soln, using methyl red indicator, 2.22(i). Add 0.5 ml excess  $\text{HCl}$  and concentrate by heating to volume of 400 ml. If cloudiness appears, it is imperative to remove  $\text{SiO}_2$  by evaporation and dehydration below  $120^\circ$ . If soln is perfectly clear, heat to boiling, add slowly 10 ml of 5%  $\text{BaCl}_2$  soln, and allow to stand overnight. Filter on dense filter paper, place paper in Pt crucible, and ignite in electric furnace. Cool in desiccator, and weigh as  $\text{BaSO}_4$ . To insure against possible inclusion of  $\text{SiO}_2$ , add 2 drops of  $\text{HF}$  and 1 drop of  $\text{H}_2\text{SO}_4$  (1+1), heat carefully, reignite, and weigh. Report as percentage of S or  $\text{SO}_2$ .

## PHOSPHORUS

## 1.22

*Sodium Peroxide Method*

Place 10 g of  $\text{Na}_2\text{O}_2$  in either iron or porcelain crucible and thoroly mix with it 5 g of the prepared soil, 1.2(a). If soil has a low organic matter content, add a little starch to hasten reaction. Heat mixture carefully by applying flame of Bunsen burner directly upon surface of charge and sides of crucible until reaction starts; cover crucible and keep at low red heat 30 min. Do not allow fusion. By means of large funnel and stream of hot  $\text{H}_2\text{O}$  transfer charge to 500 ml volumetric flask, acidify with  $\text{HCl}$ , and boil. Cool, and make to mark. (If reaction has taken place properly, there should be no particles of undecomposed soil in bottom of flask.) Allow the  $\text{SiO}_2$  to settle and draw off 200 ml of clear soln.

Or, in lieu of above, oxidize 5–10 g of the material and disintegrate melt as directed under 1.20. Dissolve residue with  $\text{HNO}_3$  (1+1), dilute to 500 ml, and withdraw 200 ml aliquot.

Precipitate the Fe, Al, and P with  $\text{NH}_4\text{OH}$  (1+1), filter, and wash several times with hot  $\text{H}_2\text{O}$ ; return precipitate to beaker, and dissolve in hot  $\text{HCl}$  (1+4), pouring the acid upon filter to dissolve any precipitate remaining. Evaporate soln and washings to complete dryness on water bath. Take up with  $\text{HNO}_3$  (1+4), heating if necessary, and filter to remove  $\text{SiO}_2$ . Evaporate filtrate and washings to ca 10 ml and add 2 ml of  $\text{HNO}_3$ . Neutralize excess of acid with the  $\text{NH}_4\text{OH}$  and add  $\text{HNO}_3$  until soln becomes clear, avoiding an excess. Heat to 40–50° in water bath, add 15 ml of molybdate soln, 2.7(a), and keep at this temp. 1–2 hours. Let stand overnight, filter, and wash free from acid with cold  $\text{H}_2\text{O}$ . Transfer filter to beaker and dissolve in standard  $\text{NaOH}$  or  $\text{KOH}$  soln (1 ml = 0.5 mg of  $\text{P}_2\text{O}_5$ ). Titrate excess of alkali with standard  $\text{HNO}_3$ , using phenolphthalein indicator. Or, after addition of 15 ml molybdate soln, allow to stand 3 hours at temp. not above 45°, filter on small filter or on Gooch crucible, and wash with 2.5%  $\text{NH}_4\text{NO}_3$  soln and then with cold  $\text{H}_2\text{O}$  until two fillings of filter do not diminish greatly color produced with phenolphthalein by 1 drop of standard alkali. Return filter and precipitate to beaker used in the precipitation of phosphomolybdate, dissolve yellow precipitate in the standard  $\text{NaOH}$  or  $\text{KOH}$  soln, add few drops of phenolphthalein indicator, 2.10(d), and titrate excess alkali with standard  $\text{HNO}_3$ . Report as percentage of  $\text{P}_2\text{O}_5$ .

## 1.23

*Magnesium Nitrate Method*

Place 5 g of prepared soil, 1.2(a), in porcelain dish. Moisten with 5–7 ml of  $\text{Mg}(\text{NO}_3)_2$  soln, 2.7(e). Dry on water bath and carefully burn organic matter. Cool, and add 10 ml of  $\text{H}_2\text{O}$ , 10 ml of  $\text{HCl}$ , and 5 ml of  $\text{HNO}_3$ . Cover dish and digest contents 2 hours on water bath, stirring 2 or 3 times during digestion. Dilute to 250 ml, mix well, and filter thru dry folded filter, pouring filtrate back thru filter until clear. Place aliquot corresponding to 2 or 4 g of soil, depending upon quantity of P present, in hard glass beaker or porcelain dish and evaporate to dryness on water bath. Take up with  $\text{HNO}_3$  (1+4), again evaporate to dryness, and heat 1 hour at 110–120°. Again take up with the dilute  $\text{HNO}_3$  and filter. Reduce combined volume of filtrates and washings to 30–40 ml. Make alkaline with  $\text{NH}_4\text{OH}$  (1+1), and dissolve precipitate with a slight excess of the dilute  $\text{HNO}_3$ . Add gradually with vigorous agitation 15 ml of molybdate soln, 2.7(a). Keep soln at 45° for an hour and then let stand overnight at room temp. Filter, and wash well with 2.5%  $\text{NH}_4\text{NO}_3$  soln and then with cold  $\text{H}_2\text{O}$ . Return filter and precipitate to precipitation flask or beaker and determine P volumetrically as directed under 1.22. Report as percentage of  $\text{P}_2\text{O}_5$ .

## 1.24

## POTASSIUM AND SODIUM (10), OR POTASSIUM ONLY

Triturate gently 0.5 or 1 g of impalpably ground soil, 1.2(b), with 1 g of dry  $\text{NH}_4\text{Cl}$  in an agate mortar, add 8 parts of  $\text{CaCO}_3$ , and mix intimately. Transfer mixture to Pt crucible, rinsing mortar with a little  $\text{CaCO}_3$ . Heat crucible gradually until fumes of  $\text{NH}_4$  salts no longer appear and lower  $\frac{1}{2}$  of crucible is brought to red heat. Maintain this temp. 40–60 min. (Temp. should be sufficient to keep the  $\text{CaCl}_2$ , formed by reaction of  $\text{NH}_4\text{Cl}$  with  $\text{CaCO}_3$ , in state of fusion. The mass does not become a melt because the fused  $\text{CaCl}_2$  is absorbed by the large quantity of  $\text{CaCO}_3$  present. If the silicate is fused by the application of excessive heat, disintegration of mass at end of operation with  $\text{H}_2\text{O}$  cannot be effected. Moreover, excessive temp. induces volatilization of alkali chlorides. The mass contracts in volume during ignition and usually is detached easily from crucible.) Transfer fused mass to porcelain dish, slake with hot  $\text{H}_2\text{O}$ , and grind thoroly with agate pestle. After washing 5 times by decantation with hot  $\text{H}_2\text{O}$ , transfer to filter and wash well (300 ml of wash  $\text{H}_2\text{O}$  is sufficient). To the filtrate add sufficient  $(\text{NH}_4)_2\text{CO}_3$  soln to precipitate any Ca present. Allow to settle, decant supernatant liquid into porcelain dish, and concentrate by evaporation, finally transferring precipitate to dish. When volume is reduced to ca 30 ml add a little  $(\text{NH}_4)_2\text{CO}_3$  and  $\text{NH}_4\text{OH}$ , heat, filter into porcelain dish, evaporate filtrate to dryness on water bath, and expel  $\text{NH}_4$  salts by ignition; or evaporate with 10 ml of  $\text{HNO}_3$ , followed by two 10 ml additions of  $\text{HCl}$  and evaporations.

If K alone is to be determined, proceed as directed under 2.42(a), beginning, "Dissolve residue in hot  $\text{H}_2\text{O}$ ." Report as percentage of  $\text{K}_2\text{O}$ .

If both K and Na are to be determined, dissolve residual alkali chlorides in 3–5 ml of  $\text{H}_2\text{O}$  (some black or brown flocculent matter usually remains undissolved), warm, and filter thru small filter into weighed Pt dish. Evaporate to dryness on water bath, carefully heat residual alkali chlorides to incipient fusion, cool, and weigh as Na and K chlorides. Dissolve combined chlorides in 30 ml of  $\text{H}_2\text{O}$ , add 1.5 ml of  $\text{H}_2\text{PtCl}_6$  soln, 2.40(b), evaporate to sirupy consistency, and cool. Add 15 ml of 80% alcohol, triturate residue, and filter thru asbestos Gooch. Continue the washing with the alcohol until all traces of Pt soln are removed, and make certain that the precipitate is transferred completely on the filter. Dry Gooch 1 hour in electric oven and weigh. Dissolve  $\text{K}_2\text{PtCl}_6$  with hot  $\text{H}_2\text{O}$ , wash with 80% alcohol, and again place in drying oven for 1 hour. Cool in desiccator, weigh, and calculate to  $\text{K}_2\text{O}$ . Calculate to  $\text{KCl}$  and deduct from combined weight of Na and K chlorides to obtain  $\text{NaCl}$ . Report as percentage of  $\text{Na}_2\text{O}$ .

## 1.25

## ARSENIC (11)

Weigh 5 g of air-dried soil and transfer to 200 ml Kjeldahl flask. Add 20 ml of  $\text{H}_2\text{SO}_4$  (arsenic free) and thoroly mix acid with soil by rotating flask. Add 5 ml of  $\text{HNO}_3$  (arsenic free) and 0.1 g of  $\text{KClO}_3$  to  $\text{H}_2\text{SO}_4$  soil mixture in flask. Heat flask gently at first, then gradually increase heat until the soln boils, and digest at this temp. until all organic matter is oxidized and acid soln is clear. (For soils of high organic matter content, it may be necessary to repeat addition of  $\text{HNO}_3$  to oxidize C and obtain clear soln.) Cool flask. Dilute soln with 50 ml of  $\text{H}_2\text{O}$  and concentrate by boiling until  $\text{SO}_2$  fumes are given off; repeat operation twice to expel all traces of oxides of N. Dilute soln in flask with  $\text{H}_2\text{O}$ , transfer to 100 ml volumetric flask, cool, and make to mark. Take an aliquot and determine arsenic by modified Gutzeit method, 29.1.

## SELENIUM (12)

## 1.26

## I. For soils

Pulverize air-dried sample with wooden roller until all portions other than rock fragments are disintegrated. Separate rock fragments by use of 2 mm sieve. Sub-

sample sieved material to secure representative sample of 50 g. Transfer weighed material to distilling flask equipped with short condenser and thistle safety tube, all glass connections (Fig. 4). Add 100 ml of HBr to which has been added 2 ml of Br. Warm gently 15 min. and distil into 100 ml Erlenmeyer flask containing 5 ml of  $H_2O$ . Have outlet of distilling tube submerged. If, on gentle warming, a drop of Br does not collect beneath  $H_2O$  in receiver, add 2 ml of Br to distilling flask thru thistle tube and repeat gentle warming. Distil 60 ml into receiver. To distillate in Erlenmeyer add 25 ml of  $H_2O$  and cool in iced  $H_2O$ . Pass slow stream of  $SO_2$  into distillate until the Br is removed. Add 0.25 g of  $NH_2OH \cdot HCl$ . Warm Erlenmeyer

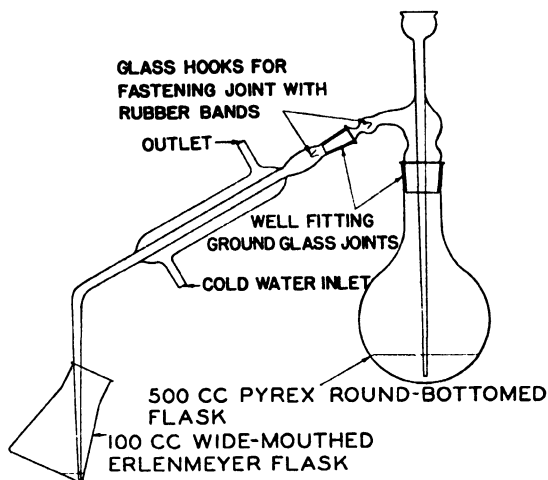


FIG. 4.—APPARATUS FOR DETERMINATION OF SELENIUM IN SOILS

on steam bath at  $80^\circ$  15 min. and allow to stand overnight. The Se will appear at bottom of Erlenmeyer as rose-pink precipitate. Modify further treatment according to quantity of precipitate, as directed below.

(a) *Precipitate not greater than 0.5 mg.*—Filter Se precipitate thru small asbestos Gooch crucible with suction. If small quantity of oily material accompanies precipitated Se, wash asbestos pad with 10 ml of alcohol and then with 10 ml of  $H_2O$ . Redissolve precipitated Se from asbestos pad with 10 ml of 48% HBr, which has been rendered bright red by addition of Br. Collect filtrate by suction in 25 ml volumetric flask and wash pad with 2 portions of  $H_2O$ . Decolorize filtered soln with  $SO_2$  and add 1 ml of a soln containing 100 mg of  $NH_2OH \cdot HCl$  and 25 mg of gum arabic/ml. Make to volume with  $H_2O$ . Transfer flask and contents to steam bath and warm at  $80^\circ$  for 30 min.; cool to room temp., shake vigorously, and transfer to a 50 ml Nessler tube. Before final precipitation of the Se in volumetric flask prepare series of standards in 25 ml volumetric flasks by addition of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, and 0.7 mg of Se as  $Na_2SeO_4$ . Precipitate these standards after addition of HBr, Br,  $H_2O$ ,  $NH_2OH \cdot HCl$ , and gum arabic, and treat precisely as sample is treated. Compare sample with standard in any suitable color comparator and determine quantity of Se. Express results as p.p.m. of air-dried soil.

(b) *Precipitate below 1 p.p.m. (established by preceding determination) and for greater precision.*—Distil 100 g of sample with HBr, 1.26. When distillation is complete, replace sample in distillation flask with a second 100 g sample. Add to distillate from first distillation, 50 ml additional HBr and 2–4 ml of Br, together with 22



ml of  $\text{H}_2\text{SO}_4$ . Allow to stand and repeat distillation as often as necessary to integrate the minute quantities of Se until an adequate quantity for measurement is obtained.

(c) *Initial precipitate in excess of 0.5 mg.*—Redissolve washed precipitate in HBr, colored with Br as directed under (a). Transfer dissolved material to a 100 ml beaker and dilute with 20% HBr to volume of 50 ml. Precipitate this soln with  $\text{SO}_2$  and add 0.25 g of  $\text{NH}_4\text{OH} \cdot \text{HCl}$ . Warm on steam bath 15 min. and allow to stand overnight at room temp. Filter on weighed Gooch, dry 4 hours at  $85^\circ$ , and weigh. (Balance used must be sensitive to at least 0.05 mg.)

## 1.27

## II. For shales and sulfide ores

Place 200 ml of  $\text{HNO}_3$  in 400 ml porcelain evaporating dish and heat to gentle boiling. Add slowly 10 g of the powdered sample. If ensuing reaction is vigorous, add sample in small quantities and allow reaction to subside after each addition. After entire sample has been added, add 50 ml of  $\text{H}_2\text{SO}_4$  and allow mixture to stand on water bath overnight. Transfer to distillation flask and proceed as directed in 1.26, 1.26(a), or 1.26(b). If shales are free from sulfides, treat as prescribed in 1.26.

## TOTAL AND AVAILABLE BORON (15)

## 1.28

## REAGENTS

- (a) *Sulfuric acid soln.*—Approximately 4.0 *N*, containing 110 ml of acid/liter.
- (b) *Sulfuric acid soln.*—Approximately 0.36 *N*, containing 10 ml of acid/liter.
- (c) *Calcium hydroxide soln.*—Saturated. Filter just before using.
- (d) *Potassium carbonate soln.*—40 g/100 ml of  $\text{H}_2\text{O}$ .
- (e) *Standard boron soln.*—2.857 g of  $\text{H}_3\text{BO}_3$ /liter of  $\text{H}_2\text{O}$ . (This soln contains 0.5 mg of B/ml.) Prepare working standards by diluting suitable portions of this soln as described below.
- (f) *Quinalizarin soln.*—Dissolve 0.005 g of quinalizarin in 1 liter of 98.0%  $\text{H}_2\text{SO}_4$ .

## 1.29

## PREPARATION OF STANDARD CURVE

Dilute 20 ml of the B soln to 1000 ml with  $\text{H}_2\text{O}$  (Soln I, which contains 0.01 mg of B/ml). Dilute 100 ml of Soln I to 1000 ml with  $\text{H}_2\text{O}$  and designate as Soln II. Using these solns, measure series of aliquots covering the range 0.0002–0.003 mg into soft glass test tubes (ca  $22 \times 150$  mm inside dimensions, a size needed to facilitate proper mixing). Adjust volume to exactly 1 ml with  $\text{H}_2\text{O}$  and add 10 ml of the quinalizarin soln. Stopper at once and mix by gentle shaking, but do not allow reagent to come in contact with rubber stopper. Allow tubes to stand until cooled to  $25^\circ$ , transfer soln to photoelectric colorimeter tubes, and with the appropriate filter in place make readings. Plot scale readings against B concentration on graph paper and connect points with smooth curve.

## 1.30

## DETERMINATION

(a) *Total boron.*—Fuse 0.5 g of soil with 3 g of anhydrous  $\text{Na}_2\text{CO}_3$  in Pt crucible. Cool, and place crucible in 250 ml beaker containing ca 50 ml of  $\text{H}_2\text{O}$ . Place cover-glass on beaker and add ca 4 *N*  $\text{H}_2\text{SO}_4$  from time to time until melt has disintegrated and soln has pH of 5.5–6.0. Transfer resultant solns to 500 ml volumetric flask. Wash beaker and crucible several times with  $\text{H}_2\text{O}$  and add washings to flask. (Total volume of soln now should not exceed 150 ml.) Make to volume with methyl or ethyl alcohol, and mix contents thoroly. Allow to stand and then decant liquid thru filter or clarify by centrifuging.

Place 400 ml aliquot of the clear soln in 600 ml beaker (B-free glass) and add 100–150 ml of  $H_2O$  to prevent subsequent precipitation. Add  $K_2CO_3$  until soln is alkaline, evaporate to small volume, and transfer to Pt dish. Evaporate to dryness and ignite at temp. not exceeding  $450^\circ$ , just enough to destroy organic matter. After cooling, add 4 ml of the 0.36  $N$   $H_2SO_4$ , and triturate thoroly with a policeman. Place 1 ml aliquot of this soln in one of soft glass tubes, add 10 ml of the quinalizarin- $H_2SO_4$  soln, stopper tube, and mix thoroly by shaking gently. Cool to  $25^\circ$ , transfer soln to colorimeter tube, and make reading as directed for standards. Calculate B content of aliquot and report result in p.p.m. of total B.

(b) *Available boron*.—Place 20 g sample of soil (air-dried, 20-mesh) in 125 ml flask (B-free glass), add 40 ml of  $H_2O$ , and then attach reflux condenser. (1 drop of tributyl citrate prevents foaming.) Boil 5 min., disconnect condenser, and after cooling to room temp. filter suspension at once with Büchner or ordinary funnel. (Clarification may be facilitated by adding not more than 0.05 g of  $CaCl_2 \cdot 2H_2O$ .) Place 20 ml of the clear extract in Pt dish and add 5 drops of the  $K_2CO_3$  soln, or place in a porcelain evaporating dish and add 2 ml of the saturated  $Ca(OH)_2$  soln. Evaporate to dryness and ignite gently at temp. not over  $450^\circ$  to destroy nitrates and all organic matter. After cooling, add 5 ml of the 0.36  $N$   $H_2SO_4$  and triturate thoroly with a policeman. Filter thru 9 cm paper, and place 1 ml aliquot of filtrate in soft glass tube. Add exactly 10 ml of the quinalizarin- $H_2SO_4$  soln. Stopper tube and mix thoroly by shaking gently. Cool to  $25^\circ$  and proceed as directed in (a). Report result as p.p.m. of available B.

#### FLUORINE (14)

##### 1.31

##### REAGENTS

- (a) *Calcium peroxide or hydroxide*.—Of known F content.
- (b) *Phenolphthalein soln*.—0.1% in 1 + 1 alcohol.
- (c) *Sodium alizarin sulfonate soln*.—0.05% aqueous soln.

##### 1.32

##### DETERMINATION

For soils of high F content, use 0.5 g charge; for those low in F, use 1 g. Mix charge intimately with 3 times its weight of the  $CaO_2$  or  $Ca(OH)_2$  in either Ni or Pt crucible. Char thoroly below  $500^\circ$ , and then heat at  $900^\circ$  for 30 min. Cool, and transfer the incinerate into distillation flask devoid of silica coating. Wash walls of flask with 5 ml of  $H_2O$ ; add 3 drops of the phenolphthalein soln; neutralize with 60%  $HClO_4$ , and then add 15 ml more. Connect distillation flask with condenser and bring suspension to  $135 \pm 5^\circ$ ; maintain distillation temp. and volume during collection of 200–250 ml of distillate, while passing current of steam thru suspension. Make distillate to 250 ml.

Use 25 ml aliquot, diluted with equal volume of alcohol. Introduce 10 drops of the sodium alizarin sulfonate soln; neutralize with 0.05  $N$   $NaOH$  and then adjust pH to 3.0,  $\pm 0.2$ , with 2.5 ml of 0.05  $N$   $HCl$ . Titrate with 0.01  $N$   $Th(NO_3)_4$  to faint pink end point corresponding to that of blank. Express results as p.p.m. of F.

#### REPLACEABLE BASES IN SOILS DEVOID OF CARBONATES

##### 1.33

##### REAGENTS

(a) *Neutral normal ammonium acetate soln*.—Prepare ca 2  $N$   $NH_4OH$  from C. P.  $NH_4OH$  and 2  $N$  acetic acid from acetic acid. Mix the two solns and adjust to pH 7.0 by means of bromothymol blue or by glass electrode. Keep in stoppered bottle.

(b) *Sodium cobaltinitrite soln.*—Dissolve 30 g of  $\text{Na}_2\text{CO}(\text{NO}_2)_6$  in 100 ml of  $\text{H}_2\text{O}$ , and filter thru asbestos, (d), on Gooch crucible. Keep in glass-stoppered bottle at  $10^\circ$ .

(c) *Acetic acid.*—Approximately 0.15 N. Dilute 9 ml of 99% acetic acid to 1000 ml with  $\text{H}_2\text{O}$ .

(d) *Asbestos.*—Digest ca 24 hours  $\text{HNO}_3$  (1+9) with enough  $\text{KMnO}_4$  to maintain deep purple color.

(e) *Sodium hydroxide soln.*—10 g of NaOH in 90 ml of  $\text{H}_2\text{O}$ .

#### DETERMINATIONS

##### 1.34

##### *Total Replaceable Bases*

Weigh 10 g of 0.5 mm air-dried soil into 250 or 300 ml Pyrex Erlenmeyer flask; introduce 100 ml of the  $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$  soln, stopper, and shake vigorously 2–3 seconds. Let stand 1 hour or longer, agitating every 15 min. Filter on 70 mm Büchner filter with light suction. Transfer soil from flask to filter and leach with the  $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$  soln to total volume of 250 ml. Transfer filtrate to 400 ml Pyrex beaker and save residue for determination of exchange capacity. Evaporate filtrate and bake to dryness on hot plate. Ignite organic matter in electric furnace 15 min. at  $550^\circ$ .

Dissolve residue with excess of 0.1 N HCl and add 2 drops of methyl red soln, avoiding undue excess of the HCl by letting the system digest slowly on hot plate in covered beaker; add more HCl if necessary, as indicated by change in the methyl red. (Dissolution is complete when liquid appears free of solids other than fine particles of C.) Filter off insoluble matter and wash beaker and filter 5–6 times. Back titrate with 0.1 N NaOH to distinct yellow, and record net acid in terms of 0.1 N. On basis of 10 g charge, each ml net acid used expresses 1 milliequivalent replaceable bases/100 g of soil.

##### 1.35

##### *Replaceable Calcium*

To soln from 1.34 add 10 ml of HCl, make ammoniacal with  $\text{NH}_4\text{OH}$  (1+1), and determine Ca as directed under 1.14(c). Each ml of 0.1 N  $\text{KMnO}_4$  = 1 milliequivalent of Ca/100 g of soil.

##### 1.36

##### *Replaceable Potassium (15)*

Evaporate filtrate from 1.35 on hot plate until crust begins to form. Add 15 ml of  $\text{HNO}_3$ , cover beaker, and evaporate to dryness on hot plate. Wash cover-glass and sides of beaker and again evaporate to dryness. Wash down all traces of  $\text{NH}_4$ -salts condensation, add 1–2 drops of the NaOH, and evaporate to dryness. Cool, and add 20 ml of acetic acid. Police bottom and sides of beaker; filter, and wash 3 times with 5 ml of  $\text{H}_2\text{O}$ . Cool to at least  $10^\circ$  and add, with stirring, 5 ml of the Na cobaltinitrite soln. Allow to stand overnight at temp. below  $10^\circ$ . While cold, filter thru asbestos, 1.33(d), on 15 ml Gooch crucible. Rinse beaker and wash precipitate 5 times with ice-cold  $\text{H}_2\text{O}$ . Remove crucible and wipe outside free of any adhering reagent. Place crucible in original beaker and just cover with hot  $\text{H}_2\text{O}$ . Add few drops of 0.05 N  $\text{KMnO}_4$  from buret and then introduce 5 ml of  $\text{H}_2\text{SO}_4$  (1+4). Titrate with the  $\text{KMnO}_4$ , timing stirring with reduction of the  $\text{KMnO}_4$  to maintain a slight excess during this step. Remove crucible, rinse with hot  $\text{H}_2\text{O}$ , place beaker over low flame, and digest near boiling 2–3 min. Assure that  $\text{KMnO}_4$  continues in slight excess. Remove beaker from flame, add enough 0.05 N oxalic acid to discharge purple color, and complete titration with the  $\text{KMnO}_4$ . Based on 10 g charge, 1 ml of 0.05 N  $\text{KMnO}_4$  = 0.0003259 g of K, or 0.08 milliequivalent/100 g of soil.

## 1.37

## Replaceable Magnesium

(a) *Determination by difference (applicable only to soils of meager Na content characteristic of humid regions).*—Subtract the sum of Ca, 1.35, and K, 1.36, milliequivalents from total milliequivalents of replaceable bases. Difference = replaceable Mg.

(b) *Direct determination.*—Evaporate filtrate from 1.35 to crust formation on hot plate. Add 15 ml of  $\text{HNO}_3$ , cover beaker, and evaporate to dryness. Add 10 ml of  $\text{HCl}$  (1+1), warm 5 min. on hot plate, and wash cover and sides of beaker. Make slightly ammoniacal, heat to boiling, add 0.2–0.3 g of  $(\text{NH}_4)_2\text{S}_2\text{O}_8$ , and digest on hot plate to flocculate Mn, maintaining alkalinity by frequent dropwise additions of  $\text{NH}_4\text{OH}$  (1+1) to maintain slight ammoniacal odor. Filter on 7 cm filter, rinse beaker, and wash filter 3–4 times with hot slightly ammoniacal  $\text{NH}_4\text{Cl}$  (2%). To filtrate add 2 ml of 1 M citric acid and 10 ml of 10%  $(\text{NH}_4)_2\text{HPO}_4$  soln and also add sufficient  $\text{NH}_4\text{OH}$  to make alkaline to methyl red. Allow to stand 20 min., add  $\text{NH}_4\text{OH}$  ( $\frac{1}{2}$  volume), stir until precipitate appears, and allow to stand overnight. Filter on 7 cm paper; rinse beaker 3 times and wash filter 4 times with dilute alcohol (1+1). Remove, open, and place filter against side of beaker; wash precipitate from filter to total volume of ca 50 ml; add 1 drop of 1% bromocresol green soln, and deliver from buret, with stirring, enough 0.1 N  $\text{H}_2\text{SO}_4$  to impart a permanent yellow color. Titrate with 0.1 N  $\text{NaOH}$  to first shade of blue. Each ml of net acid = 0.00202 g of  $\text{MgO}$ , or 1 milliequivalent  $\text{Mg}/100$  g of soil, basis of 10 g charge.

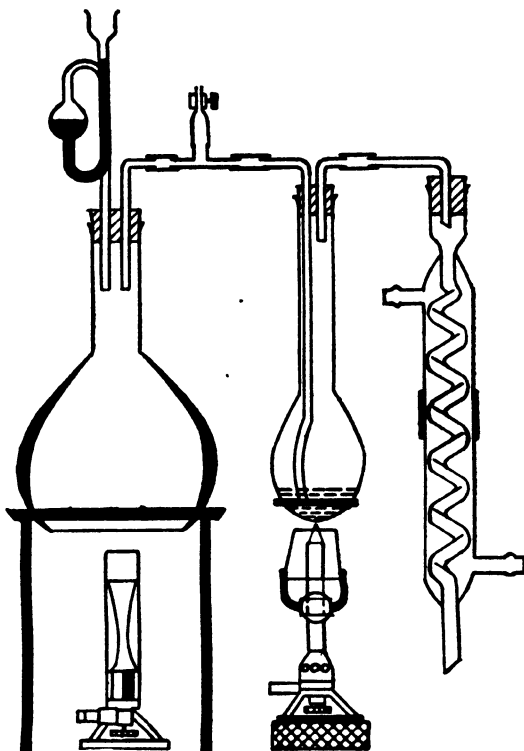


FIG. 5—STEAM DISTILLATION APPARATUS

## AVAILABLE AND REPLACEABLE BASES IN CALCAREOUS SOILS

## Total Available Bases

## 1.38

## APPARATUS

Steam distillation apparatus, Fig. 5, consisting of 3 liter insulated Pyrex boiling flask, 500 ml Pyrex long-necked Kjeldahl flask, 14" coiled condenser, and connec-

tions of 7 mm glass tubing. T-tube connecting flasks is for release of steam pressure. (Beveled tip of condenser should extend to bottom of receiver.)

## 1.39

## DETERMINATION

Weigh 10 g of 0.5 mm air-dried soil into Kjeldahl flask; introduce 100 ml of 2 *N*  $\text{NH}_4\text{Cl}$ , connect to steam digestion apparatus (Fig. 5), and continue distillation until 100 ml distillate titrates only 1.2 ml of 0.1 *N*  $\text{NH}_4\text{OH}$ . Maintain heat under digestion flask so that volume shall not exceed 100 ml. Remove flask and rinse connecting tubes into flask; cool, add 100 ml of  $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$  soln, 1.33(a), and 5 drops of  $\text{NH}_4\text{OH}$  (1 + 1), and shake gently. Filter on 70 ml Büchner filter. Use the  $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$  soln to transfer soil onto filter, and wash residue with additional 100 ml. Transfer filtrate to 400 ml Pyrex beakers and save residue for exchange capacity determination. Evaporate filtrate on hot plate until crust appears to form on side of beaker. Remove from hot plate, add 25 ml of  $\text{HNO}_3$ , place cover-glass over beaker, and continue evaporation to dryness. Wash sides of beaker and dissolve residue by warming on hot plate. Add, while swirling, 5 ml of 10% oxalic acid. Evaporate to dryness on steam bath or below 100° on hot plate. If inside of beaker is not frosted, add 5 ml more oxalic acid and again evaporate. Wash sides of beaker and again evaporate to dryness. Ignite in muffle furnace at 550° or over Bunsen burner, taking care that heat has been applied to all spots where precipitation occurred.

Proceed as directed in 1.34, beginning "Dissolve residue," and report as "total available bases."

## 1.40

*Available Calcium.*—See 1.35

## 1.41

*Replaceable Potassium.*—See 1.36

## 1.42

*Available Magnesium.*—See 1.37

## 1.43

*Replaceable Calcium*

Determine by difference, as follows:

(a) If total available Mg is comparatively low, subtract the carbonate equivalence, 1.6 or 1.7, from available Ca, 1.40, and designate difference as replaceable Ca.

(b) If available Mg approximates Ca, subtract one-half of carbonate equivalence from total available Ca, 1.40, and designate difference as replaceable Ca.

## 1.44

*Replaceable Magnesium*

(a) If condition 1.43(a) obtains, replaceable Mg = available Mg.

(b) If condition 1.43(b) obtains, subtract one-half of carbonate equivalence, 1.43(b), and designate difference as replaceable Mg.

## EXCHANGE CAPACITY

## 1.45

## REAGENTS

(a) *Alcohol.*—U.S.P. 95%. Should react alkaline to phenolphthalein upon addition of 0.1 ml of 0.1 *N*  $\text{NaOH}$ /100 ml.

(b) *Hydrochloric acid.*—0.1 *N*. Delivered from automatic zero-point buret fitted into acid reservoir.

(c) *Methyl orange soln.*—0.1% aqueous soln.

(d) *Anti-foam.*—Mixture of mineral oil and capryl alcohol.

## 1.46

## APPARATUS

Same as 1.38, except that stopper fitted to Kjeldahl flask is provided with a small separator for introduction of alkali. Wash the Kjeldahl flask and its connections free of  $\text{NH}_4$  salts immediately before use.

## 1.47

## DETERMINATION

Wash residue from 1.34 or 1.39 with alcohol in portions of 15 ml to total of 250 ml. Drain free of alcohol and transfer immediately to distillation flask. Add few drops of the anti-foam and connect flask with steam distillation apparatus (Fig. 5). Place receiver containing 10 ml of the HCl and 2 drops of the methyl orange soln under condenser. Dip the condenser outlet into the acid in receiver, start steam passage thru soil suspensions, and continue until all air is displaced from distillation flask. Introduce 12–15 ml of ca normal NaOH into the separator. Relieve steam pressure by opening at T, and deliver the alkali slowly from the separator into distillation flask without admitting air. Close steam line and continue distillation. Stir acid in receiver frequently during the first minute or two to assure that ammoniacal liquor does not rise to surface. Add additional quantity of the HCl if indicator shows insufficiency. Continue distillation until 200 ml of distillate is collected. Complete titration by first adding enough of the NaOH to make the distillate distinctly alkaline, and finish titration to first change from clear yellow. Ml of acid used/10 g charge = milliequivalents of absorbed  $\text{NH}_4$ /100 g soil, or *exchange capacity*.

## 1.48

## REPLACEABLE HYDROGEN

Obtain by subtracting replaceable bases, 1.34, or the sum of 1.35, 1.36, and 1.37, from absorbed  $\text{NH}_4$ , 1.47.

## 1.49

## QUALITATIVE TEST FOR SOIL REACTION

Place strips of neutral litmus paper in bottom of a number of Petri dishes; over these lay 1 or 2 thicknesses of filter paper (free from acid); and place prepared soil, 1.2(a), on filter paper. With horn spoon or clean spatula press soil down firmly against paper and add enough  $\text{H}_2\text{O}$  (tested and found neutral) to saturate soil. Cover dishes, allow to stand 30 min., and note color of test paper. Have a check Petri dish containing neutral litmus paper and filter paper, moistened with the same  $\text{H}_2\text{O}$ , stand under same conditions. (Filter paper gives a uniform background and evenness of contact.)

## HYDROGEN-ION CONCENTRATION

## 1.50

## DETERMINATION

(a) *Electrometric*.—Weigh 20–25 g of soil into 50 ml beaker and add boiled  $\text{H}_2\text{O}$  carefully until soil is soft enough to admit ready penetration of electrodes. (Resultant moisture content is slightly above the moisture equivalent and well below the water-holding capacity of the soil. The mass may be stirred with glass rod to attain a uniform mass.) Tap beaker gently on table top, press glass electrode and its companion calomel electrode into soil, and make the pH reading. Make several readings on each sample, withdrawing electrodes and pressing them again into soil mass. Initial reading is often inaccurate, since complete equilibrium is not always attained by the first contact between the electrodes and the soil mass.

(b) *Colorimetric*.—Determine reaction values on fresh moist samples, using soil-to-water ratio of 1:5, with intermittent agitation for 30 min. (16).

## 1.51

## METHOD OF STATEMENT

For simplicity and ease of interpretation use a dual system of statement, giving both pH values and their equivalents in arithmetically related numbers in parentheses, as tabulated.

SØRENSEN OR pH VALUES	SPECIFIC ACIDITY	SØRENSEN OR pH VALUES	SPECIFIC ALKALINITY
7.0	0.0	7.0	0.0
6.9	0.5	7.1	0.5
6.8	1.0	7.2	1.0
6.7	1.5	7.3	1.5
6.6	2	7.4	2
6.5	3	7.5	3
6.4	4	7.6	4
6.3	5	7.7	5
6.2	6	7.8	6
6.1	8	7.9	8
6.0	10	8.0	10
5.9	12.5	8.1	12.5
5.8	16	8.2	16
5.7	20	8.3	20
5.6	25	8.4	25
5.5	31.5	8.5	31.5
5.4	40	8.6	40
5.3	50	8.7	50
5.2	63	8.8	63
5.1	80	8.9	80
5.0	100	9.0	100
4.9	125	9.1	125
4.8	160	9.2	160
4.7	200	9.3	200
4.6	250	9.4	250
4.5	315	9.5	315

## 1.52

## NITRATE NITROGEN

Place 100 g of prepared air-dried soil, 1.2(a), and 500 ml of H<sub>2</sub>O in suitable container, and agitate 5 min. Add 1 g of CaO or 2 g of precipitated CaCO<sub>3</sub>, agitate thoroly, allow to stand 10–20 min., and obtain a clear filtrate. If filtrate contains 6 p.p.m. of Cl, or less, proceed as directed under 37.17, using 25 ml of filtrate; if it contains more than 6 p.p.m. of Cl, proceed as directed under 37.19, using 25 ml or volume containing N not in excess of 0.1 mg of N in form of nitrate. Report as percentage of nitrate N.

## 1.53

## ALKALI SALTS

To 100 g of soil in 500 ml flask, add 250 ml of H<sub>2</sub>O. Stopper, shake thoroly, and allow to stand overnight. Filter thru Pasteur-Chamberland tube. Evaporate 50 ml of filtrate (corresponding to 20 g of sample) to dryness in Pt dish on steam bath, ignite gently to decompose organic matter, cool in desiccator, and weigh for total salts. Dissolve residue in the Pt dish in 10–15 ml of hot H<sub>2</sub>O, transfer to 50 ml volumetric flask, cool, and dilute to mark.

For determination of Cl, titrate an aliquot of 10 ml against 0.1 N AgNO<sub>3</sub> soln. Report as percentage of NaCl.

For determination of alkali carbonate, titrate aliquot of 10 ml against 0.1 N HCl. Report as percentage of Na<sub>2</sub>CO<sub>3</sub>.

Determine sulfates by difference. Should much CaSO<sub>4</sub> be present, filter 10 ml

aliquot of the 50 ml soln of the salts thru small filter, add 75 ml of alcohol, and digest 3 hours; filter, wash with alcohol, and ignite; subtract amount of  $\text{CaSO}_4$  from total quantity of sulfates.

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## 2. FERTILIZERS\*

### 2.1

#### DIRECTIONS FOR SAMPLING (1)—OFFICIAL

Use the slotted single-tube, the slotted double-tube, or the slotted tube and rod, all with pointed ends.

Each official sample shall consist of at least 1 lb. of material taken in following manner: Remove a core from top to bottom of bag. Take cores from not less than 10% of bags present unless this process necessitates cores from more than 20 bags, in which case take core from 1 bag for each additional ton represented. If less than 100 bags, sample not less than 10 bags; if less than 10 bags, sample all bags. Thoroughly mix portions taken on clean oilcloth or paper, reduce by quartering to quantity of sample required, and place in air-tight container.

### 2.2

#### PREPARATION OF SAMPLE (2)—OFFICIAL

Pass entire sample thru 10-mesh sieve before subdividing for analysis. Reduce gross sample by quartering to quantity sufficient for analytical purposes. For fertilizer materials and moist fertilizer mixtures, grind to pass a sieve with 1 mm circular openings, or 20-mesh Tyler standard sieve; for dry mixtures that show a tendency to segregate grind to pass a 35-mesh Tyler standard sieve. Grind as rapidly as possible to avoid loss or gain of moisture during operation. Mix thoroughly and preserve in tightly stoppered bottles.

### 2.3

#### MECHANICAL ANALYSIS OF BONE, TANKAGE, AND BASIC SLAG—OFFICIAL

Transfer 100 g of original bone or tankage or 10 g of basic slag to sieve having circular openings 0.5 mm in diam. Sift, breaking lumps by means of soft rubber pestle if material has tendency to cake. Weigh coarse portion remaining on sieve. Determine fine portion by difference.

#### MOISTURE

### 2.4

#### *By Drying—Official*

(Not applicable to samples containing compounds other than  $H_2O$  that are volatilized at the temperature of drying.)

Heat 2 g of prepared sample, 2.2, for 5 hours in water oven at temp. of boiling  $H_2O$  (98–100°). In case of potash salts,  $NaNO_3$ , and  $(NH_4)_2SO_4$ , heat at ca 130° to constant weight. Report percentage loss in weight as moisture.

#### *By Distillation with Toluene (3)—Tentative*

(Applicable to urea, calcium nitrate, ammonium nitrate, and other salts containing water of crystallization, or that are volatile or decomposed at low temperature, and to mixtures containing such salts.)

### 2.5

#### APPARATUS

(a) *Distillation apparatus.*—250 ml Pyrex Erlenmeyer flask, receiving tube or trap graduated in 0.1 ml, and condenser. Ground-glass joints are preferable, but pressed cork may be used. See 27.4.

(b) *Tube brush attached to a long wire.*

\* Volumetric flasks used in fertilizer determinations should have a maximum diameter at the capacity mark not exceeding the limits set by National Bureau of Standards Circ. 9 (1916); namely, 13 mm for 200 ml flasks, 15 mm for 250 ml flasks, and 18 mm for 500 ml flasks.

## 2.6

## DETERMINATION

Place in flask sufficient sample to give 2–5 ml of  $H_2O$  (weight should not exceed 20 g). Weigh rapidly to 1 mg. (Extremely hygroscopic materials should be placed in covered weighing tubes, and samples should be weighed out by difference.) If sample is likely to bump, add enough dry sand to cover bottom of flask. Add 100 ml of toluene immediately and connect flask to trap and condenser. Pour 50 ml of toluene thru condenser, filling trap. Bring mixture to boil and distil slowly (ca 2 drops/second), until most of  $H_2O$  has passed over, then increase rate of distillation to ca 4 drops 1 second. When all  $H_2O$  has apparently distilled, wash down condenser by pouring 5–10 ml of toluene in at top, and continue distillation until no more  $H_2O$  will distil over. (Distillation for organic materials and salts that contain no water of crystallization may be completed within an hour, but such salts as  $Ca(NO_3)_2$  require much longer time, 7–10 hours.) If  $H_2O$  remains in condenser, remove by brushing down with tube brush attached to long wire, washing down condenser at same time with toluene. Allow receiving tube to come to room temp. Force any drops adhering to sides of tube into  $H_2O$  column with a rubber band wrapped around a wire. Read volume of  $H_2O$  and calculate percentage of sample, assuming weight of 1 ml of  $H_2O$  to be 1 g at room temp.

It is necessary to have condenser and receiving tube absolutely clean at start to prevent adherence of  $H_2O$  to glass. Clean with chromic-sulfuric acid, rinse with alcohol, and dry thoroly. Used toluene may be recovered by distillation from anhydrous  $CuSO_4$ .

## TOTAL PHOSPHORIC ACID

*Gravimetric Method—Official*

## 2.7

## REAGENTS

(a) *Molybdate soln.*—Dissolve 100 g of  $MoO_3$  in a mixture of 144 ml of  $NH_4OH$  and 271 ml of  $H_2O$ . Cool, and pour soln slowly and with constant stirring into a cool mixture of 489 ml of  $HNO_3$  and 1148 ml of  $H_2O$ . Keep final mixture in a warm place for several days or until portion heated to  $40^\circ$  deposits no yellow precipitate of  $NH_4$  phosphomolybdate. Decant soln from any sediment and preserve in glass-stoppered vessels.

(b) *Ammonium nitrate soln.*—Dissolve 100 g of phosphate-free  $NH_4NO_3$  in  $H_2O$  and dilute to 1 liter.

(c) *Magnesia mixture.*—(1) Dissolve 11 g of  $MgO$  in  $HCl$  (1+4), avoiding an excess of the acid; add a little  $MgO$  in excess; boil few minutes to precipitate  $Fe$ ,  $Al$ , and  $P_2O_5$ ; and filter. To filtrate add 140 g of  $NH_4Cl$  and 130.5 ml of  $NH_4OH$  and dilute to 1 liter. Or, (2) dissolve 55 g of crystallized  $MgCl_2 \cdot 6H_2O$  in  $H_2O$ , add 140 g of  $NH_4Cl$  and 130.5 ml of  $NH_4OH$ , and dilute to 1 liter. Or, (3) dissolve 55 g of crystallized  $MgCl_2 \cdot 6H_2O$  in  $H_2O$ , add 140 g of  $NH_4Cl$ , and dilute to 870 ml. Add  $NH_4OH$  to each required portion of soln just before using, at rate of 15 ml per 100 ml of soln.

(d) *Ammonium hydroxide soln for washing.*—(1+9). Should contain not less than 2.5% of  $NH_3$  by weight.

(e) *Magnesium nitrate soln.*—Dissolve 150 g of  $MgO$  in  $HNO_3$  (1+1), avoiding an excess of acid; add a little  $MgO$  in excess, boil, filter from excess of  $MgO$ ,  $Fe_2O_3$ , etc., and dilute to 1 liter.

## 2.8

## PREPARATION OF SOLUTION

Treat 2 g of sample by one of methods given below. Cool soln, dilute to 200 ml, mix, and pour on dry filter.

(a) Dissolve in 30 ml of  $\text{HNO}_3$  and 3–5 ml of  $\text{HCl}$  and boil until organic matter is destroyed. (Suitable for materials containing small quantity of organic matter.)

(b) Dissolve in 15–30 ml of  $\text{HCl}$  and 3–10 ml of  $\text{HNO}_3$ . (Recommended for fertilizers containing much  $\text{Fe}$  or  $\text{Al}$  phosphate and for basic slag.)

(c) Evaporate with 5 ml of the  $\text{Mg}(\text{NO}_3)_2$  soln, ignite, and dissolve in  $\text{HCl}$ . (Suitable for organic material like cottonseed meal alone or in mixtures.)

(d) Boil with 20–30 ml of  $\text{H}_2\text{SO}_4$  in 200 ml flask, adding 2–4 g of  $\text{NaNO}_3$  or  $\text{KNO}_3$  at beginning of digestion and small quantity after soln has become nearly colorless, or adding the nitrate in small portions from time to time. When soln is colorless, add 150 ml of  $\text{H}_2\text{O}$  and boil few minutes. (Generally applicable to materials or mixtures containing large quantities of organic matter. With cottonseed meals and materials of like nature it is best to add first ca 5 ml of  $\text{HNO}_3$  and then the  $\text{H}_2\text{SO}_4$ .) Before adding the nitrate, allow mixture to digest, at a gentle heat if necessary, until violence of reaction is over.

## 2.9

## DETERMINATION

Pipet aliquot of prepared soln corresponding to 0.25 g, 0.50 g, or 1 g, into a 250 ml beaker; add  $\text{NH}_4\text{OH}$  in slight excess, and barely dissolve precipitate formed with few drops of  $\text{HNO}_3$ , stirring vigorously. If  $\text{HCl}$  or  $\text{H}_2\text{SO}_4$  has been used as solvent, add ca 15 g of crystalline  $\text{NH}_4\text{NO}_3$  or a soln containing that quantity. To hot soln add 70 ml of the molybdate soln for every decigram of  $\text{P}_2\text{O}_5$  present. Digest at ca  $65^\circ$  for 1 hour, and determine whether or not the  $\text{P}_2\text{O}_5$  has been completely precipitated by adding more molybdate soln to clear supernatant liquid. Filter, and wash with cold  $\text{H}_2\text{O}$  or preferably with the  $\text{NH}_4\text{NO}_3$  soln. Dissolve precipitate on filter with  $\text{NH}_4\text{OH}$  (1+1) and hot  $\text{H}_2\text{O}$  and wash into beaker to volume of not more than 100 ml. Neutralize with  $\text{HCl}$ , using litmus paper or bromothymol blue as indicator; cool; and from buret add slowly (ca 1 drop/second), stirring vigorously, 15 ml of the magnesia mixture for each decigram of  $\text{P}_2\text{O}_5$  present. After 15 min. add 12 ml of  $\text{NH}_4\text{OH}$ . Let stand until supernatant liquid is clear (usually 2 hours), filter, wash precipitate with  $\text{NH}_4\text{OH}$  (1+9) until the washings are practically free from chlorides, dry, burn at low heat, and ignite to constant weight, preferably in electric furnace, at  $950\text{--}1000^\circ$ ; cool in desiccator, and weigh as  $\text{Mg}_2\text{P}_2\text{O}_7$ . Report as percentage of  $\text{P}_2\text{O}_5$ .

With basic slag dehydrate aliquot (20 ml) of prepared soln by evaporating to dryness on steam or hot water bath. Treat with 5 ml of  $\text{HCl}$  and 25 ml of hot  $\text{H}_2\text{O}$ , digest in order to complete the soln, and filter off  $\text{SiO}_2$ . Proceed as above. Before precipitating with magnesia mixture, add 5 ml of 5%  $\text{Na}$  acetate soln.

*Volumetric Method (4)—Official*

## 2.10

## REAGENTS

(a) *Molybdate soln.*—To 100 ml of molybdate soln, 2.7(a), add 5 ml of  $\text{HNO}_3$ . Filter this soln immediately before using.

(b) *Standard sodium or potassium hydroxide soln.*—Dilute 323.81 ml of  $N$  alkali, free from carbonates, to 1 liter; 100 ml of the soln should neutralize 32.38 ml of  $N$  acid; 1 ml = 1 mg or 1% of  $\text{P}_2\text{O}_5$  on basis of 0.1 g of substance. For basic slag standardize against a standard phosphate material of about same composition as sample under examination. (Burets in constant use are likely to become so corroded as to increase their capacity and therefore should be tested at least once a year.)

(c) *Standard acid soln.*—Prepare soln of  $\text{HCl}$  or of  $\text{HNO}_3$ , corresponding to strength of (b), or to  $\frac{1}{2}$  of this strength, and standardize by titration against that soln, using the phenolphthalein indicator.

(d) *Phenolphthalein indicator*.—Dissolve 1 g of phenolphthalein in 100 ml of alcohol.

## 2.11

## PREPARATION OF SOLUTION

Treat 2 g of sample as directed under 2.8(a), (b), (c), or (d), preferably (a) when these acids are a suitable solvent, and dilute to 200 ml with  $H_2O$ .

## 2.12

## DETERMINATION

(a) For percentages up to 5 use an aliquot corresponding to 0.4 g of substance; for percentages between 5 and 20 use an aliquot corresponding to 0.2 g of substance; and for percentages above 20 use an aliquot corresponding to 0.1 g of substance. Add 5–10 ml of  $HNO_3$ , depending on method of soln (or equivalent in  $NH_4NO_3$ ); add  $NH_4OH$  until precipitate that forms dissolves only slowly on stirring vigorously, dilute to 75–100 ml, and adjust to temp. of 25–30°. If the sample is of such nature that it will not give a precipitate with  $NH_4OH$  as a test of neutralization, make the soln slightly alkaline to litmus with  $NH_4OH$  and then slightly acid with dilute  $HNO_3$ . For percentages below 5, add 20–25 ml of the freshly filtered molybdate soln; for percentages between 5 and 20, add 30–35 ml of the molybdate soln; and for percentages greater than 20, add sufficient molybdate soln to insure complete precipitation. Place soln in shaking or stirring apparatus and shake or stir 30 min. at room temp., decant *at once* thru filter, and wash precipitate twice by decantation with 25–30 ml portions of  $H_2O$ , agitating thoroly and allowing to settle. Transfer precipitate to filter and wash with cold  $H_2O$  until filtrate from 2 fillings of filter yields pink color upon addition of phenolphthalein and 1 drop of the standard alkali. Transfer precipitate and filter to the beaker or precipitating vessel, dissolve precipitate in small excess of the standard alkali, add few drops of phenolphthalein indicator, and titrate with the standard acid.

(b) *Not applicable to superphosphate and other fertilizers that contain sulfates* (5).—Proceed as directed under (a) to point where soln is diluted to 75–100 ml. Heat in water bath to 45–50°, add the molybdate soln at rate of 75 ml for each decigram of  $P_2O_5$  present, and allow mixture to remain in bath 30 min., stirring occasionally. Decant *at once* thru filter, wash, and titrate as directed under (a).

## WATER-SOLUBLE PHOSPHORIC ACID

## 2.13

*Gravimetric Method—Official*

Place 1 g of sample on 9 cm filter and wash with successive small portions of  $H_2O$  until filtrate measures ca 250 ml. Allow each portion of wash  $H_2O$  to pass thru filter before adding more and use suction if the washing would not otherwise be complete within 1 hour. If filtrate is turbid, add 1–2 ml of  $HNO_3$ . Dilute to convenient volume, mix well, and proceed as directed under 2.9.

## 2.14

*Volumetric Method—Official*

Treat sample as directed under 2.13. To aliquot of soln corresponding to 0.1, 0.2, or 0.4 g, add 10 ml of  $HNO_3$ , nearly neutralize with  $NH_4OH$ , dilute to 60 ml, and proceed as directed under 2.12.

## CITRATE-INSOLUBLE PHOSPHORIC ACID—OFFICIAL

## 2.15

## REAGENTS

*Ammonium citrate soln* (6).—Should have a sp. gr. of 1.09 at 20° and pH of 7.0 as determined by electrometric method with hydrogen electrode or by colorimetric method with phenol red. When using colorimetric method proceed as follows:

Dissolve 370 g of crystallized citric acid in 1500 ml of  $H_2O$  and nearly neutralize by adding 345 ml of  $NH_4OH$  (28–29%  $NH_3$ ). If concentration of  $NH_3$  is less than 28%, add correspondingly larger volume and dissolve the citric acid in correspondingly smaller volume of  $H_2O$ . Cool, and make exactly neutral as follows:

Transfer 10 ml of the citrate soln to standard test tube of a hydrogen-ion comparator set with color standards and add 0.5 ml of 0.02% phenol red soln or sufficient volume to give same concentration of indicator as used in color standards. Add from graduated pipet a few drops of  $NH_4OH$  (1+7), mix, compare color by use of comparator with that of color standards of same indicator, add more  $NH_4OH$  if necessary, and repeat test until color matches that of color standard corresponding to pH of 7.0. If the  $NH_4OH$  added is in excess of that required to give pH of 7.0, repeat test, using smaller quantity of  $NH_4OH$ . From quantity of  $NH_4OH$  required to produce in sample a color that exactly matches standard, calculate quantity of  $NH_4OH$  required to neutralize soln. Add this quantity of  $NH_4OH$  and check pH of soln by repeating test as before with the addition of a small quantity of  $NH_4OH$  or of a citric acid soln as may be required. When color matches, dilute soln, if necessary, to density of 1.09 at 20°. (Volume will be ca 2 liters.) Keep in tightly stoppered bottles and check pH from time to time.

Phenol red is recommended in place of bromothymol blue as salt effect due to presence of  $NH_4$  citrate soln gives pH reading with latter indicator that is ca 0.20 unit too high. When bromothymol blue is used, subtract 0.20 from observed reading

The other reagents and solns are described under 2.7 and 2.10.

## 2.16

### DETERMINATION (7)

(a) *Acidulated samples*.—After washing out water-soluble  $P_2O_5$ , 2.13, transfer filter and residue, within period not to exceed an hour, to 250 ml flask containing 100 ml of the  $NH_4$  citrate soln previously heated to 65° in water bath. Close flask tightly with a smooth rubber stopper and shake vigorously until filter paper is reduced to a pulp, relieving pressure by momentarily removing stopper. Loosely stopper flask to prevent evaporation and return it to the bath. Maintain contents of flask at exactly 65°, keeping level of  $H_2O$  in bath above that of the citrate soln in flask. Shake flask every 5 min. At expiration of exactly 1 hour from time filter and residue were introduced, remove flask from bath and immediately filter contents as rapidly as possible thru Whatman filter paper No. 5 or other paper of equal speed and retentiveness. (It is recommended that filtration be made with suction and use of Büchner funnel or ordinary funnel with Pt or other cone.) Wash with  $H_2O$  at 65° until volume of filtrate is ca 350 ml, allowing time for thoro draining before adding new portions of  $H_2O$ . If sample gives a cloudy filtrate, wash with 5%  $NH_4NO_3$  soln. Determine  $P_2O_5$  in citrate-insoluble residue by one of following methods: (1) Dry filter and contents, transfer to crucible, ignite until all organic matter is destroyed, and digest with 10–15 ml of HCl until all phosphate is dissolved; (2) transfer wet filter with contents to 200 ml flask, add 30–35 ml of  $HNO_3$  and 5–10 ml of HCl, and boil until all phosphate is dissolved; or (3) treat filter and contents as directed under 2.8(c) or (d). Dilute soln to 200 ml, mix well, filter thru a dry filter, and proceed as directed under 2.9 or 2.12.

(b) *Non-acidulated samples other than basic slag*.—Place 1 g of sample on 9 cm filter paper. Without previous washing with  $H_2O$ , proceed as directed under (a) and determine  $P_2O_5$  as directed under 2.9 or 2.12. If substance contains much animal matter (bone, fish, etc.), dissolve residue insoluble in  $NH_4$  citrate by one of processes described under 2.8(c) or (d).

**2.17 CITRATE-SOLUBLE AND AVAILABLE PHOSPHORIC ACID—OFFICIAL**

Subtract sum of water-soluble and citrate-insoluble  $P_2O_5$  from total to obtain the citrate-soluble  $P_2O_5$ . Subtract citrate-insoluble  $P_2O_5$  from total to obtain chemically available  $P_2O_5$  in acidulated samples, dicalcium phosphate, precipitated bone phosphate, and precipitated bone.

**CITRIC ACID-SOLUBLE PHOSPHORIC ACID IN BASIC SLAG (8)***Gravimetric Method—Official***2.18****PREPARATION OF SOLUTION**

Weigh 5 g of prepared slag, 2.2, into a 500 ml cylindrical shaking flask (Wagner) containing 5 ml of alcohol. (Neck of flask should be at least 22 mm wide, and graduation marks at least 8 cm below mouth.) Make up to mark with 2% citric acid soln at 17.5°. Fit flask with rubber stopper and place at once in rotary apparatus, shaking flask 30 min. at 30–40 r.p.m. Filter immediately on dry filter and analyze soln at once.

**2.19****DETERMINATION**

To 50 ml of the clear filtrate in beaker add 100 ml of molybdate soln, 2.7(a), and place beaker in water bath; when temp. of contents reaches 65°, remove beaker and cool to room temp. Filter, and wash yellow precipitate of  $NH_4$  phosphomolybdate 4 or 5 times with 1%  $HNO_3$ . Dissolve precipitate in 100 ml of cold 2%  $NH_4OH$ , and nearly neutralize with  $HCl$ . Add to soln dropwise, with continuous stirring, 15 ml of magnesia mixture, 2.7(c), and proceed as directed under 2.9.

**2.20***Volumetric Method—Official*

In an aliquot of the clear soln, 2.18, determine  $P_2O_5$  as directed under 2.12.

**2.21****DETECTION OF NITRATES—OFFICIAL**

Mix 5 g of fertilizer with 25 ml of hot  $H_2O$ , and filter. To 1 volume of this soln add 2 volumes of  $H_2SO_4$ , free from  $HNO_3$  and oxides of N, and allow mixture to cool. Add few drops of concentrated soln of ferrous sulfate in such manner that fluids will not mix. If nitrates are present, junction shows at first purple, afterwards brown color, or if only minute quantity is present, a reddish color. To another portion of soln add 1 ml of 1%  $NaNO_3$  soln and test as before to determine whether sufficient  $H_2SO_4$  was added in first test.

**ORGANIC AND AMMONIACAL NITROGEN ONLY****2.22****REAGENTS**

For ordinary work 0.5 N acid is recommended, but in determining very small quantities of N, 0.1 N acid is recommended. In titrating mineral acids against  $NH_4OH$  use cochineal or methyl red as indicator.

(a) *Standard hydrochloric acid.*—Proceed as directed under 43.7 and 43.8, or determine absolute strength as follows:

**PRELIMINARY TEST:** Place a measured portion of the acid in Erlenmeyer flask and add excess of  $CaCO_3$  to neutralize free acid and few drops of 10%  $K_2CrO_4$  soln as indicator. Titrate with 0.1 N  $AgNO_3$  soln and note exact quantity required to precipitate the chlorides.

**FINAL DETERMINATION:** To a measured portion of the acid add from buret 1 drop

in excess of required quantity of  $\text{AgNO}_3$  soln as determined by preliminary test. Heat mixture to boiling, protect from light, and allow to stand until precipitate is granular. Filter on Gooch crucible, previously heated to  $140\text{--}150^\circ$  and weighed; wash with hot  $\text{H}_2\text{O}$ , testing filtrate to verify an excess of  $\text{AgNO}_3$ . Dry the  $\text{AgCl}$  at  $140\text{--}150^\circ$ , cool, and weigh.

(b) *Standard sulfuric acid*.—The acids may be standardized by any of the official methods in Chap. 43. Or, determine strength of acid by precipitation with  $\text{BaCl}_2$  soln as follows: Dilute measured quantity of the acid to ca 100 ml; heat to boiling; and add, dropwise, 10%  $\text{BaCl}_2$  soln until no further precipitation occurs. Continue boiling ca 5 min., allow to stand 5 hours or longer in a warm place, pour supernatant liquid on weighed Gooch crucible or ashless filter, treat precipitate with 25–30 ml of boiling  $\text{H}_2\text{O}$ , transfer to filter, and wash with boiling  $\text{H}_2\text{O}$  until filtrate is free from chlorides. Dry, ignite over a Bunsen burner, and weigh as  $\text{BaSO}_4$ .

(c) *Standard alkali soln*.—0.1 N soln is recommended. Accurately determine strength of this soln by titration against the standard acid prepared as directed under (a) or (b), or proceed as directed in any official method under Chap. 43.

(d) *Sulfuric acid*.—Should contain 93–96%  $\text{H}_2\text{SO}_4$  and be free from nitrates and  $(\text{NH}_4)_2\text{SO}_4$ .

(e) *Metallic mercury, or mercuric oxide*.—Hg or  $\text{HgO}$  of reagent grade, free from N.

(f) *Sulfide, or thiosulfate soln*.—Dissolve 40 g of commercial  $\text{K}_2\text{S}$  in 1 liter of  $\text{H}_2\text{O}$ . A soln of 40 g of  $\text{Na}_2\text{S}$  or 80 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in a liter may be used.

(g) *Sodium hydroxide soln*.—Dissolve ca 450 g of commercial  $\text{NaOH}$ , free from nitrates, in 1 liter of  $\text{H}_2\text{O}$ . (A soln having sp. gr. of 1.36 or higher may be used.)

(h) *Cochineal indicator*.—Digest 3 g of pulverized cochineal in mixture of 50 ml of alcohol and 200 ml of  $\text{H}_2\text{O}$  1 or 2 days at ordinary temp., agitating frequently, and filter.

(i) *Methyl red indicator*.—Dissolve 1 g of methyl red in 200 ml of alcohol.

Test reagents before using by blank determination with sugar, which insures partial reduction of any nitrates present.

## 2.23

### APPARATUS

(a) *Kjeldahl flasks for digestion and distillation*.—Total capacity ca 550 or 800 ml. Made of hard, moderately thick, and well-annealed glass.

(b) *Distillation flask*.—Use any suitable flask of ca 550 or 800 ml capacity, fitted with rubber stopper thru which passes lower end of Kjeldahl connecting bulb to prevent  $\text{NaOH}$  being carried over mechanically during distillation. Use bulb 5 or 6 cm in diam., and connect upper end of bulb tube to condenser tube by means of rubber tubing.

## 2.24

### Kjeldahl Method—Official

Place 0.7–3.5 g, according to N content of material to be analyzed, in a digestion flask. Add ca 0.7 g of  $\text{HgO}$ , or its equivalent in metallic Hg, and 20–30 ml of  $\text{H}_2\text{SO}_4$  (0.1–0.3 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  may also be used in addition to the Hg, or in many cases, in place of it). Place flask in inclined position and heat below boiling point of acid until frothing has ceased. (A small piece of paraffin may be added to prevent extreme foaming.) Increase heat until acid boils briskly and digest for a time after mixture is colorless or nearly so, or until oxidation is complete (ca 2 hours).

After cooling, dilute with ca 200 ml of  $\text{H}_2\text{O}$ , and add a few pieces of granulated Zn or pumice stone to prevent bumping, and 25 ml of the  $\text{K}_2\text{S}$  or  $\text{Na}_2\text{S}$  or  $\text{Na}_2\text{S}_2\text{O}_3$  soln with shaking. (If  $\text{Na}_2\text{S}_2\text{O}_3$  is to be used, it should first be mixed with the  $\text{NaOH}$  so

that they may be added together. When no Hg or HgO is to be used the addition of  $K_2S$  or  $Na_2S$  or  $Na_2S_2O_3$  soln is unnecessary.) Add sufficient NaOH soln to make reaction strongly alkaline (50 ml usually sufficient), pouring it down side of flask so that it does not mix at once with the acid soln. Connect flask to condenser by means of Kjeldahl connecting bulb, taking care that tip of condenser extends below surface of the standard acid in receiver; mix contents by shaking and distil until all  $NH_3$  has passed over into a measured quantity of the standard acid. (First 150 ml of distillate generally contains all the  $NH_3$ .) Titrate with standard alkali soln, using the methyl red or cochineal indicator.

**2.25***Gunning Method—Official*

Place 0.7–3.5 g, according to N content of material to be analyzed, in a digestion flask. Add 10 g of powdered  $K_2SO_4$  or anhydrous  $Na_2SO_4$  and 15–25 ml (ordinarily ca 20 ml) of  $H_2SO_4$  (0.1–0.3 g of crystallized  $CuSO_4$  may also be added). Conduct digestion as in Kjeldahl process, starting with temp. below boiling point and increasing heat gradually until frothing ceases. Digest for a time after mixture is colorless or nearly so, or until oxidation is complete (usually 2 hours). Complete as directed under 2.24, but do not add  $K_2S$  or  $Na_2S$  or  $Na_2S_2O_3$ . In making mixture alkaline before distilling add litmus paper or a few drops of phenolphthalein indicator. (Pink color given by phenolphthalein, indicating an alkaline reaction, is destroyed by excess of strong fixed alkali.)

**2.26***Kjeldahl-Gunning-Arnold Method—Official*

Place 0.7–3.5 g, according to N content of material to be analyzed, in a digestion flask. Add 15–18 g of  $K_2SO_4$  or anhydrous  $Na_2SO_4$ , 1 g of  $CuSO_4$  or ca 0.7 g of HgO (or its equivalent in metallic Hg), and 25 ml of the  $H_2SO_4$ . Heat mixture gently until frothing ceases, then boil briskly, and continue digestion for a time after mixture is colorless or nearly so, or until oxidation is complete (ca 2 hours). Cool, add ca 200 ml of  $H_2O$ , and if HgO or metallic Hg has been used, add also 50 ml of the  $K_2S$  or  $Na_2S$  or  $Na_2S_2O_3$  soln. Make strongly alkaline with the NaOH soln and proceed as directed under 2.24.

**TOTAL NITROGEN****2.27***Kjeldahl Method Modified to Include Nitrogen of Nitrates—Official*

Place 0.7–3.5 g, according to N content of material to be analyzed, in Kjeldahl digestion flask. (1) Add 30 ml of the  $H_2SO_4$  containing 1 g of commercial salicylic acid, shake until *thoroly* mixed, allow to stand at least 30 min. with frequent shaking or until complete soln results, and then add 5 g of  $Na_2S_2O_3 \cdot 5H_2O$  and digest as directed below; or, (2) add to substance 30 ml of  $H_2SO_4$  containing 2 g of the salicylic acid, allow to stand at least 30 min. with frequent shaking or until complete soln results, and then add gradually 2 g of Zn dust (an impalpable powder—granulated Zn or filings not satisfactory), shaking contents of flask at same time, and digest as follows:

Heat over low flame until all danger from frothing has passed. Increase heat until acid boils briskly and continue boiling until white fumes no longer escape from flask (5–10 min.). Add ca 0.7 g of HgO, or its equivalent in Hg, and continue boiling until liquid in flask is colorless, or nearly so. If contents of flask are likely to become solid before this point is reached, add 10 ml more of  $H_2SO_4$ . Complete determination as directed under 2.24. Test reagents by blank determinations.



**2.28** *Gunning Method Modified to Include Nitrogen of Nitrates—Official*

Place 0.7–3.5 g, according to N content of material to be analyzed, in digestion flask; add 30 ml of  $\text{H}_2\text{SO}_4$  containing 1 g of commercial salicylic acid; shake until thoroly mixed; and allow to stand, shaking frequently, at least 30 min., or until complete soln results. Add 5 g of  $\text{Na}_2\text{S}_2\text{O}_3$  and heat soln 5 min.; cool, add 10 g of  $\text{K}_2\text{SO}_4$  or anhydrous  $\text{Na}_2\text{SO}_4$ , heat very gently until foaming ceases, and proceed with digestion as directed under 2.25.

**AMMONIACAL NITROGEN****2.29** *Magnesium Oxide Method—Official*

Place 0.7–3.5 g, according to  $\text{NH}_3$  content of material to be analyzed, in a distillation flask with ca 200 ml of  $\text{H}_2\text{O}$  and 2 g or more of  $\text{MgO}$  free from carbonates. Connect flask to condenser by means of Kjeldahl connecting bulb, distil 100 ml of liquid into measured quantity of standard acid, and titrate with standard alkali, using cochineal or methyl red indicator, 2.22(h) or (i).

**NITRATE AND AMMONIACAL NITROGEN****2.30** *Ferrous Sulfate-Zinc-Soda Method—Official*

(Not applicable in presence of organic matter, calcium cyanamide, and urea.)

Place 0.35, 0.5, or 0.7 g of sample in 600–700 ml flask and add 200 ml of  $\text{H}_2\text{O}$ , 5 g of powdered Zn, 1–2 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , and 50 ml of  $\text{NaOH}$  soln (sp. gr. 1.33). Connect flask with the distilling apparatus, distil, collect distillate in usual way in standard acid, and titrate with standard alkali, using cochineal or methyl red indicator, 2.22(h) or (i). In the analysis of nitrate salts dissolve 3.5 or 5.0 g in  $\text{H}_2\text{O}$ , make up to 250 ml, and use a 25 ml aliquot.

**2.31** *Devarda Method (9)—Official*

(Not applicable in presence of organic matter, calcium cyanamide, and urea.)

Place 0.35 or 0.5 g of sample in a 600–700 ml flask and add 300 ml of  $\text{H}_2\text{O}$ , 3 g of Devarda alloy, and 5 ml of  $\text{NaOH}$  soln (42% by weight), pouring latter down side of flask so that it does not mix at once with contents. Connect, by means of Davisson (10) or other suitable scrubbing bulb that will prevent passing over of any portion of spray, with condenser, the tip of which always extends beneath surface of standard acid in receiving flask. Mix contents of distilling flask by rotating. Heat slowly at first and then at such a rate that the 250 ml of distillate required will pass over in 1 hour. Collect distillate in measured quantity of standard acid, 2.22(a) or (b), and titrate with standard alkali soln, 2.22(c), using cochineal or methyl red indicator, 2.22(h) or (i).

In the analysis of nitrate salts, dissolve 3.5 or 5.0 g in  $\text{H}_2\text{O}$ , make up to 250 ml, and use a 25 ml aliquot.

**NITRATE NITROGEN****2.32** *Robertson Method (11)—Official*

(Applicable in presence of calcium cyanamide and urea)

(a) Determine total N as directed under 2.27 or 2.28.

(b) Determine water-insoluble N as directed under 2.35, but use 2.5 g of the sample. Make filtrate up to 250 ml.

(c) Determine ammoniacal N in 50 ml of filtrate as directed under 2.29.

(d) Place another 50 ml portion of filtrate in 500 ml Kjeldahl flask and add 2 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  and 20 ml of  $\text{H}_2\text{SO}_4$ . (If total N is over 5%, use 5 g of  $\text{FeSO}_4$ .) Digest over hot flame until all  $\text{H}_2\text{O}$  is evaporated and white fumes appear and continue digestion at least 10 min. to drive off nitrate N. If severe bumping occurs, add 10–15 glass beads. Add 0.65 g of Hg, or its equivalent of  $\text{HgO}$ , and digest until all the organic matter is oxidized. Cool, dilute, add the  $\text{K}_2\text{S}$  soln, and complete as directed under 2.24. Add a pinch of mixture of Zn dust and granular Zn (20-mesh) to each flask before distillation to prevent bumping.

Total N(a) – water-insoluble N(b) = water-soluble N. Water-soluble N – N obtained in (d) = nitrate N.

Ammoniacal N + nitrate N = mineral N. Total N – mineral N = organic N.

### 2.33 *Jones Modification of Robertson Method (12)—Official*

(Applicable when determination of water-soluble nitrogen is not needed.)

Weigh 0.5 g of sample into Kjeldahl flask, add 50 ml of  $\text{H}_2\text{O}$ , and rotate gently. Add 2 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  and rotate. Add 20 ml of  $\text{H}_2\text{SO}_4$ . Digest over hot flame. When  $\text{H}_2\text{O}$  is evaporated and white fumes appear, add 0.65 g of Hg and proceed as directed under 2.24. Cool, dilute, and distil as usual. Total N – N thus found = nitrate N.

### 2.34 WATER-INSOLUBLE NITROGEN IN CYANAMIDE (13)—OFFICIAL

Weigh 2 g of finely ground cyanamide and place in mortar. Gradually add ca 70 ml of  $\text{H}_2\text{O}$  while stirring with pestle and grind thoroly. Transfer mixture to beaker, washing out mortar with  $\text{H}_2\text{O}$ . Filter on 11 cm paper. When all cyanamide has been transferred to the paper, wash with an additional 250 ml of  $\text{H}_2\text{O}$ , allowing time for complete drainage before adding more  $\text{H}_2\text{O}$ . Remove filter paper and residue to digestion flask. Determine insoluble N in residue as directed under 2.24, 2.25, or 2.26.

### 2.35 WATER-INSOLUBLE ORGANIC NITROGEN—OFFICIAL

Place 1 or 1.4 g of the material in a 50 ml beaker, wet with alcohol, add 20 ml of  $\text{H}_2\text{O}$ , and allow to stand 15 min., with occasional stirring. Transfer supernatant liquid to 11 cm Whatman No. 2 filter paper, and wash residue 4 or 5 times by decantation with  $\text{H}_2\text{O}$  at room temp. (20–25°). Use long-stemmed funnels 2.5" in diam. and having an angle of 60°. Finally transfer all residue to filter paper and complete washing until filtrate measures 250 ml. Dry, and determine N in residue as directed under 2.24 or 2.25.

## NITROGEN ACTIVITY

### 2.36 *Removal of Water-Soluble Nitrogen—Official*

(a) *Mixed fertilizers*.—Place the quantity of material equivalent to 50 mg of water-insoluble organic N, 2.35, on 11 cm filter paper wet with alcohol, and wash with  $\text{H}_2\text{O}$  at room temp. until filtrate measures 250 ml. If material is oily or does not wet readily with  $\text{H}_2\text{O}$ , wash with 5 ml of alcohol and then with requisite quantity of  $\text{H}_2\text{O}$ . If necessary to use 4 g or more of the material, weigh required quantity into a small beaker, wet with alcohol, wash by decantation, finally transfer to filter, and finish extraction as directed previously.

(b) *Raw materials*.—Place the quantity of material equivalent to 50 mg of water-insoluble N, 2.35, in small mortar; add ca 2 g of powdered rock phosphate, mix thoroly, transfer to filter paper, wet with alcohol, and wash with  $\text{H}_2\text{O}$  at room temp.

until filtrate measures 250 ml. If material is oily or does not wet readily with  $\text{H}_2\text{O}$ , wash with 5 ml of alcohol and then with requisite quantity of  $\text{H}_2\text{O}$ .

### 2.37 *Water-Insoluble Organic Nitrogen Soluble in Neutral Permanganate—Official*

Using 25 ml of tepid  $\text{H}_2\text{O}$ , transfer insoluble residue obtained in 2.36 to 400 ml Griffin low-form beaker; add 1 g of  $\text{Na}_2\text{CO}_3$ , mix, and add 100 ml of 2%  $\text{KMnO}_4$  soln. Cover with watch-glass and immerse 30 min. in steam or hot water bath, keeping liquid in beaker below that of  $\text{H}_2\text{O}$  in bath. Stir twice at intervals of 10 min. At end of 30 min. remove from bath, add immediately 100 ml of cold  $\text{H}_2\text{O}$ , and filter thru heavy 15 cm folded filter. Wash with small quantities of cold  $\text{H}_2\text{O}$  until filtrate measures ca 400 ml. Determine N in residue and filter as directed under 2.24 or 2.25, correcting for N contained in filter. The N thus obtained is the inactive water-insoluble organic N. The N obtained under 2.35—percentage of N found = water-insoluble organic N soluble in neutral permanganate.

### *Water-Insoluble Organic Nitrogen Distilled from Alkaline Permanganate (14)—Official*

#### 2.38

#### REAGENTS

(a) *Stock soln of potassium permanganate.*—Dissolve 50 g of  $\text{KMnO}_4$  in liter of  $\text{H}_2\text{O}$ . Dissolve 0.5 g of Na oxalate in 300 ml of  $\text{H}_2\text{O}$  and 10 ml of  $\text{H}_2\text{SO}_4$ . Heat to  $75\text{--}80^\circ$  and titrate with the  $\text{KMnO}_4$  soln, using a Mohr pipet or an all-glass buret to contain permanganate soln.  $235.89 \div \text{result of titration in ml} = \text{concentration of } \text{KMnO}_4 \text{ in g/liter}$ . Adjust concentration to 50 g/liter, protect from light, and store at a temp. above  $15^\circ$ .

(b) *Stock soln of sodium hydroxide.*—Dissolve 300 g of NaOH in 1 liter of  $\text{H}_2\text{O}$ . Cool before using.

(c) *Alkaline permanganate soln.*—Mix equal quantities of stock solns (a) and (b) and add 10 ml of  $\text{H}_2\text{O}$  for each liter of soln that mixture is calculated to make. Use this soln immediately, as it is unstable.

#### 2.39

#### DETERMINATION

Dry residue remaining after treatment of material as directed in 2.36 at temp. not exceeding  $80^\circ$  and transfer from filter to 500–600 ml Kjeldahl distillation flask, loosening adhering particles by rubbing gently with stiff brush but avoiding transfer of portions of brush or of paper fibers. Add 20 ml of  $\text{H}_2\text{O}$ , 15–20 small glass beads or fragments of pumice stone, a drop of mineral lubricating oil weighing not more than 50 mg, and 100 ml of the alkaline permanganate soln. Connect with upright condenser to lower end of which has been attached 100 ml graduated cylinder containing standard acid and so arranged as to receive distillate below surface of acid or otherwise trapped so as to prevent loss of  $\text{NH}_3$  fumes. Digest slowly with very low flame 30 min., barely below distillation point, using coarse wire gauze and asbestos paper between flask and flame. Gradually raise temp., and after all danger from frothing has passed distil 95 ml in 60 min. ( $\pm 5$  min.), controlling distillation to obtain ca 24 ml of distillate in each 15 min. period. Conduct first part of distillation over bare flame but use wire gauze 10 min. before completion to avoid breaking flask. Transfer distillate to Erlenmeyer flask or to beaker and titrate with standard alkali, using cochineal or methyl red indicator. When a tendency to froth is noticed, lengthen digestion period, and no trouble will be experienced when distillation is begun. During digestion gently rotate flask occasionally, particularly if material shows tendency to adhere to sides.

The N thus obtained is active water-insoluble organic N. If it is found to be less

than 55% of the total water-insoluble organic N present, it is recommended that a second portion of the sample be prepared as directed under 2.36. Dry residue below 80°, transfer from filter to Kjeldahl flask as directed above, and determine N as directed under 2.24 or 2.25. Recalculate percentage of active water-insoluble N on basis of quantity of water-insoluble N thus found.

Previous to digestion with alkaline permanganate, the washed sample may be transferred from filter to flask by spreading filter on a metal disk bent to form a trough that fits the palm of the hand, brushing larger portion of material into flask with spatula, and washing in remainder with 20 ml of H<sub>2</sub>O from 20 ml pipet or small wash bottle. Do not add more H<sub>2</sub>O before digestion with alkaline permanganate, but with this exception proceed as with transfer of dried material.

### POTASH

#### *Lindo-Gladding Method (15)—Official*

### 2.40

#### REAGENTS

(a) *Ammonium chloride soln.*—Dissolve 100 g of NH<sub>4</sub>Cl in 500 ml of H<sub>2</sub>O, add 5–10 g of pulverized K<sub>2</sub>PtCl<sub>6</sub>, and shake at intervals 6–8 hours. Allow mixture to settle overnight and filter. (The residue may be used for preparation of a fresh supply.)

(b) *Platinum soln.*—Use a Pt soln containing the equivalent of 1 g of Pt (2.1 g of H<sub>2</sub>PtCl<sub>6</sub>) or a Pt soln containing the equivalent of 0.5 g of Pt (1.05 g H<sub>2</sub>PtCl<sub>6</sub>) in every 10 ml. For materials containing less than 15% of K<sub>2</sub>O, a Pt soln containing 0.2 g of metallic Pt (0.42 g of H<sub>2</sub>PtCl<sub>6</sub>) in each 10 ml is recommended.

(c) *Diglycol stearate soln.*—Dissolve 20 g of diglycol stearate tech. in 1 liter of equal parts of benzene and ethyl alcohol.

(d) *Acid alcohol.*—Mix 200 ml of 80% alcohol with 20 ml of HCl and cool to room temp.

### 2.41

#### PREPARATION OF SOLUTION

(a) *Mixed fertilizers.*—Place 2.5 g or the factor weight 2.425 g of sample in a 250 ml volumetric flask, and add 125 ml of H<sub>2</sub>O and 50 ml of saturated NH<sub>4</sub> oxalate soln, also 1 ml of the diglycol stearate soln when necessary to prevent foaming. Boil 30 min., add slight excess of NH<sub>4</sub>OH, and after cooling dilute to 250 ml, mix, and pass thru dry filter.

(b) *Potash salts (muriate and sulfate of potash, sulfate of potash and magnesia; and kainit).*—Dissolve 2.5 g or the factor weight 2.425 g and dilute to 250 ml without addition of NH<sub>4</sub>OH and NH<sub>4</sub> oxalate. When substances that interfere, such as NH<sub>3</sub>, Ca, Al, etc., are present, proceed as directed in (a).

(c) *Organic compounds (cottonseed meal, tobacco stems, etc.).*—For total K<sub>2</sub>O saturate 10 g of sample with H<sub>2</sub>SO<sub>4</sub> and ignite in muffle at low red heat to destroy organic matter. Add a little HCl, warm slightly in order to loosen mass from dish, transfer to 500 ml volumetric flask, add NH<sub>4</sub>OH and saturated NH<sub>4</sub> oxalate soln, cool, dilute to 500 ml, mix, pass thru a dry filter, and proceed as directed under 2.42(a).

(d) *Ashes from wood, cotton hulls, etc.*—Boil 10 g of sample with 300 ml of H<sub>2</sub>O 30 min., and add to hot soln a slight excess of NH<sub>4</sub>OH and then sufficient saturated NH<sub>4</sub> oxalate soln to precipitate all lime present. Cool, dilute to 500 ml, mix, and pass thru dry filter.

(e) *Nitrate of potash or nitrate of potash and soda.*—If impure, proceed as directed in (a); if sufficiently pure, proceed as directed for potash salts (b), except to evapo-

rate an aliquot to dryness in a porcelain dish with 2 ml of HCl (if Pt dish is used, add  $\text{H}_2\text{SO}_4$  instead) and to take up with  $\text{H}_2\text{O}$  and a few drops of HCl, before adding the Pt soln.

## 2.42

## DETERMINATION

(a) *Mixed fertilizers*.—In a quartz, silica, or Pt dish of ca 100 ml capacity, evaporate nearly to dryness a 25 or 50 ml aliquot of soln, 2.41(a), to which has been added sufficient K-free normal NaOH (1–2 ml) to prevent formation of free  $\text{H}_3\text{PO}_4$  during ignition; add 1 ml of  $\text{H}_2\text{SO}_4$  (1+1) and 6–8 granules of granulated sugar, evaporate to dryness, and ignite to whiteness at low temp. (The  $\text{H}_2\text{SO}_4$  may be added after evaporation to dryness and before ignition.) Maintain a dull red heat until residue is perfectly white. Dissolve residue in hot  $\text{H}_2\text{O}$ , using at least 20 ml for each decigram of  $\text{K}_2\text{O}$  present, and add a few drops of HCl and then an excess of Pt soln. Evaporate on water bath to thick paste, avoiding exposure to  $\text{NH}_3$ . Treat residue with ca 6 ml of the acid alcohol. (Temp. of wash solns should not exceed  $30^\circ$ .) After 15 min. filter on Gooch crucible or on a medium fritted crucible (Pyrex M porosity), and wash precipitate thoroly with 80% alcohol, both by decantation and on filter, continuing washing after filtrate is colorless (75 ml is usually sufficient). Wash 5 or 6 times with 10 ml portions of the  $\text{NH}_4\text{Cl}$  soln to remove impurities from precipitate. Wash again thoroly with 80% alcohol and dry precipitate 30 min. at  $100^\circ$ . Weigh and calculate to  $\text{K}_2\text{O}$ . Precipitate should be completely soluble in  $\text{H}_2\text{O}$ . If it is not, dissolve the  $\text{K}_2\text{PtCl}_6$  in hot  $\text{H}_2\text{O}$ , reweigh, and make correction for water-insoluble residue. If the factor weight and a 50 ml aliquot (containing 0.485 g of sample) are used, multiply weight by 40 to get per cent  $\text{K}_2\text{O}$ .

(b) *Muriate of potash*.—Acidify 50 ml of the soln prepared according to 2.41(b) with few drops of HCl, add 10 ml of Pt soln, and evaporate to thick paste. Treat residue as directed under (a). If  $\text{NH}_4\text{OH}$  and  $\text{NH}_4$  oxalate are used in preparation of this soln, ignite and complete determination as directed under (a).

(c) *Sulfate of potash, sulfate of potash and magnesia, and kainit*.—Acidify 50 ml of the soln prepared according to 2.41(b) with a few drops of HCl and add 15 ml of the Pt soln. Evaporate mixture and proceed as directed under (a), but use 25 ml portions of the  $\text{NH}_4\text{Cl}$  soln. If  $\text{NH}_4\text{OH}$  and  $\text{NH}_4$  oxalate are used in preparation of this soln, ignite and complete determination as directed under (a) but use 25 ml portions of  $\text{NH}_4\text{Cl}$  soln.

(d) *Ashes from wood, cotton hulls, etc.*—Prepare soln according to 2.41(d) and proceed as directed under (a) paying special attention to next to last sentence.

For conversion of  $\text{K}_2\text{PtCl}_6$  to KCl use the factor 0.30670; to  $\text{K}_2\text{SO}_4$ , 0.35843; to  $\text{K}_2\text{O}$ , 0.19376.

## WATER-SOLUBLE BORON (16)—OFFICIAL

## 2.43

## REAGENTS

(a) *Standard sodium hydroxide soln*.—Prepare ca 0.1 N soln by method given in 43.4 and 43.5, and determine exact concentration by titration against 0.1 N boric acid as directed in 2.44(a).

(b) *Methyl red indicator*.—Dissolve 0.1 g of methyl red in 50 ml of alcohol, dilute to 100 ml with  $\text{H}_2\text{O}$ , and filter if necessary.

## 2.44

## DETERMINATION

(a) *Mineral salts*.—Dissolve 5–10 g of sample in 50–75 ml of hot  $\text{H}_2\text{O}$ , decomposing carbonates, if present, with a slight excess of HCl; heat to boiling and add sufficient 10%  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  soln to precipitate sulfates, using ca 10 ml in excess. Add

in small quantities sufficient powdered  $\text{Ba}(\text{OH})_2$  to make soln alkaline, avoiding large excess; and boil ca 5 min., or until any  $\text{NH}_3$  present has been expelled. Filter into 300 ml flask, wash residue, and make filtrate acid with  $\text{HCl}$ , using an excess equivalent to a few ml of 0.1  $N$  soln. Boil, 15 min. to expel  $\text{CO}_2$ , cool by placing flask in cold  $\text{H}_2\text{O}$ , and bring to neutrality by first adding 4 or 5 drops of the methyl red indicator and then the standard  $\text{NaOH}$  soln until color of soln changes from pink to yellow. If neutral point has been exceeded, or if there is any doubt as to this, restore pink color by adding a few drops of 0.1  $N$   $\text{HCl}$  and change color to yellow again with minimum quantity of the standard  $\text{NaOH}$  soln. Add 1–2 g of neutral mannitol (mannite) and a few tenths of a ml of phenolphthalein indicator, 2.10(d), note buret reading, and again titrate soln with the standard  $\text{NaOH}$  soln until pink color develops. Add a little more mannitol and if pink color disappears continue addition of standard alkali until it reappears. Repeat procedure until addition of mannitol has no further action on end point. (If content of B in soln titrated is low, one addition of mannitol is usually sufficient.) From volume of standard alkali required in titration after addition of mannitol, corrected for volume required when running a blank, calculate quantity of B in sample: 1 ml of 0.1  $N$   $\text{NaOH}$  soln = .00108 g of B.

When an acid soln of sample gives no precipitate upon addition of a soln of  $\text{CaCl}_2$  and sufficient  $\text{NH}_4\text{OH}$  to give alkaline reaction, phosphates and Fe and Al salts are absent, and that portion of determination that involves treatment with  $\text{BaCl}_2$  and  $\text{Ba}(\text{OH})_2$  for removal of these constituents may be omitted.

(b) *Mixed fertilizers and organic compounds.*—Weigh 5 g of sample into 250 ml beaker, add 50 ml of hot  $\text{H}_2\text{O}$ , cover with watch-glass, digest 15–20 min. on water bath, filter, and wash into another beaker of same capacity. Heat filtrate to boiling and add 15 ml of 10%  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  soln, followed without undue loss of time by sufficient powdered  $\text{Ba}(\text{OH})_2$  to give an alkaline reaction as indicated by phenolphthalein; boil ca 5 min. (gently to prevent frothing over), filter, and wash. Or, if preferred, make up to mark in a volumetric flask and take an aliquot. Evaporate filtrate or aliquot to dryness in Pt or porcelain dish and ignite residue (preferably in muffle furnace) just below redness, until organic matter is completely carbonized. Treat ignited residue with hot  $\text{H}_2\text{O}$ , make slightly acid with  $\text{HCl}$ , heat nearly to boiling, make alkaline again with a slight excess of  $\text{Ba}(\text{OH})_2$ , and filter into 300 ml flask. Acidify with  $\text{HCl}$  (1+9), using excess equivalent to few ml of a 0.1  $N$  soln, boil to expel  $\text{CO}_2$ , and titrate as directed under (a).

If the  $\text{Ba}(\text{OH})_2$  has been added only in slight excess there is tendency for filtrate to become acid during evaporation with possible loss of B. It is important, therefore, that soln be kept alkaline, by repeated additions of  $\text{Ba}(\text{OH})_2$  if necessary, until evaporation is completed.

If filtrate from the  $\text{BaCl}_2$ – $\text{Ba}(\text{OH})_2$  precipitate is titrated before soluble organic matter is destroyed, the end points in the titration will usually be too indefinite to give accurate results. The purpose in evaporating filtrate and igniting residue, therefore, is to get rid of the soluble organic constituents that interfere with titration. When sample contains relatively high B content (in excess of 0.5%) a smaller sample may be taken and the quantity of organic matter present may then be too small to interfere seriously with sharpness of the end points during titration. When such is the case, boil the soln after addition of the  $\text{Ba}(\text{OH})_2$ , until any  $\text{NH}_3$  present has been expelled. Omit evaporating filtrate from  $\text{BaCl}_2$ – $\text{Ba}(\text{OH})_2$  precipitate. Add to filtrate an excess of  $\text{HCl}$  equivalent to a few ml of 0.1  $N$  soln, boil to expel  $\text{CO}_2$ , and titrate as directed under (a).

#### ACID-SOLUBLE BORON (17)—OFFICIAL

##### 2.45

##### APPARATUS

The apparatus (18) consists of two 200 ml round-bottomed flasks, Liebig condenser, and 200 ml Erlenmeyer receiving flask. One of the round-bottomed flasks,

No. 2, has a rubber stopper with two holes. Thru one hole passes a glass tube running to bottom of flask; thru other hole passes a short tube leading to condenser. The other flask, No. 1, is fitted with perforated rubber stopper and short bent tube connected by rubber tubing with the long tube in flask No. 2. The whole apparatus is supported by clamps and rings on two stands.

**2.46****DETERMINATION**

If the material to be examined is a mixed fertilizer or contains less than the equivalent of 2% of anhydrous  $\text{Na}_2\text{B}_4\text{O}_7$ , weigh 5 g into flask No. 2; if the material contains much more than 2%, use 2 g. Add 5 ml of  $\text{H}_3\text{PO}_4$  and 20 ml of methyl alcohol and connect flask with condenser. Add 100 ml of methanol (at least 95%) to flask No. 1, which is set in water bath and connected with flask No. 2. Place receiving flask in position at end of condenser and apply sufficient heat to water bath to keep a steady flow of bubbles of the methanol passing thru flask No. 2. Also apply some heat to flask No. 2 to keep volume at ca 25 ml. Continue distillation until 100 ml of distillate is obtained (ca 30 min.). Add to the distillate 2 or 3 drops of phenolphthalein indicator, 2.10(d), and 5–10 ml of 0.1 N NaOH, or enough to produce a permanent pink color. Stopper flask, shake well, and connect at once with condenser by means of Hopkins or similar bulb. Using water bath (not gas burner), distil the alcohol and use for another determination. Transfer residue, which should be not less than 10 ml, to Pt or porcelain dish, using as little  $\text{H}_2\text{O}$  as possible, and evaporate to dryness on steam or water bath. When dry, ignite below redness, acidify with few drops of ca 1 N HCl, add 20–25 ml of  $\text{H}_2\text{O}$ , and warm 1–2 min. on steam bath. Filter into small flask, thoroly wash, dilute to 50–75 ml, attach to an air-cooled condenser, and boil gently for few minutes to remove  $\text{CO}_2$ . Add 3 or 4 drops of methyl red indicator, 2.43(b), and then 0.1 N NaOH until red color just disappears. Add ca 1 g of mannitol, or less if but a small amount of B is present. (At this point, if B is present, soln will take on pinkish color; depth of color depends on quantity present, 0.01 or 0.02% usually being sufficient to give the color if soln has been carefully neutralized with the NaOH soln.) Add 2 or 3 drops of the phenolphthalein indicator and titrate with the 0.1 N NaOH. Test reagents by blank determination. (If the NaOH is free from  $\text{CO}_2$  the blank should not be more than 0.2 ml.) Calculate to B as directed under 2.44(a).

**WATER-SOLUBLE CHLORINE (19)—OFFICIAL****2.47****REAGENTS**

(a) *Standard silver nitrate soln.*—Dissolve ca 5 g of pure recrystallized  $\text{AgNO}_3$  in  $\text{H}_2\text{O}$  and dilute to 1 liter. Standardize against pure, dry NaCl and adjust so that 1 ml of soln = 0.001 g of Cl.

(b) *Potassium chromate indicator.*—Dissolve 5 g of  $\text{K}_2\text{CrO}_4$  in 100 ml of  $\text{H}_2\text{O}$ .

**2.48****DETERMINATION**

Place 2.5 g of sample on 11 cm filter paper and wash with successive portions of boiling  $\text{H}_2\text{O}$  until the washings amount to nearly 250 ml, collecting the filtrate in 250 ml volumetric flask. Cool, dilute to mark with  $\text{H}_2\text{O}$ , and mix well. Pipet 50 ml into 150 ml beaker, add 1 ml of the  $\text{K}_2\text{CrO}_4$  indicator, and titrate with the  $\text{AgNO}_3$  soln until color produced by  $\text{Ag}_2\text{CrO}_4$  appears as permanent red.

**ACID-SOLUBLE CALCIUM (#0)—TENTATIVE****2.49***Method I*

Weigh 2.5 g of sample into 250 ml volumetric flask, add 30 ml of  $\text{HNO}_3$ , and

10 ml of HCl, and boil 30 min. Cool, make to volume, and mix. Filter if necessary. Transfer to a beaker a 25 ml aliquot of dissolved sample and dilute to 100 ml. Add 2 drops of bromophenol blue indicator. Add  $\text{NH}_4\text{OH}$  (1+4) to the point where the indicator changes from yellow to green (not blue). If over-run, bring back with HCl (1+4). (This gives pH of 3.5–4.0.) Dilute to 150 ml. Bring to boiling and add slowly with constant stirring 30 ml of saturated hot  $\text{NH}_4$  oxalate soln. If the color changes from green to blue or yellow again, adjust to green with the HCl. Digest on steam bath 1 hour, or let stand overnight, and cool to room temp. Filter supernatant liquid thru quantitative filter paper on Gooch crucible or on fritted glass filter, and wash precipitate thoroly with  $\text{NH}_4\text{OH}$  (1+50). Place filter paper or crucible with precipitate in original beaker and add mixture of 125 ml of  $\text{H}_2\text{O}$  plus 5 ml of  $\text{H}_2\text{SO}_4$ . Heat to  $70^\circ$  or above and titrate with 0.1  $N$   $\text{KMnO}_4$  until first slight pink color is obtained. Correct for blank and calculate to Ca.

## 2.50

*Method II*

Place the Ca oxalate and filter paper from acid-soluble Mg, 2.51, in beaker in which precipitation was made and add a mixture of 125 ml of  $\text{H}_2\text{O}$  plus 5 ml of  $\text{H}_2\text{SO}_4$ . Heat to  $70^\circ$  or above and titrate with 0.1  $N$   $\text{KMnO}_4$  until first slight pink color appears. Correct for blank and calculate to Ca.

## ACID-SOLUBLE MAGNESIUM

## 2.51

*Gravimetric Method (21)—Official*

Weigh 2.5 g of fertilizer into 250 ml volumetric flask, add 30 ml of  $\text{HNO}_3$  and 10 ml of HCl, and boil 30 min. Cool, make to volume, and mix. Transfer an aliquot of the clear soln containing not more than 12 mg of Mg to a beaker. Partially neutralize with  $\text{NH}_4\text{OH}$ . Add a few drops of methyl red. Add  $\text{NH}_4\text{OH}$  until soln is yellow, then HCl until barely pink. Add 10 ml of saturated soln of  $\text{NH}_4$  oxalate soln for each 50 ml of soln, adjust soln to pH 5.0 (a faint pink color) by addition of HCl (1+4), or  $\text{NH}_4\text{OH}$  (1+4), boil a few minutes, cool, and again adjust reaction to pH 5.0, adding more methyl red if necessary. Stir thoroly and allow soln to stand until precipitate settles. Filter thru a 11 cm paper fine enough to retain Ca oxalate and wash 10 times with hot  $\text{H}_2\text{O}$ . Evaporate filtrate to a volume of ca 100 ml and add 5 ml of a 10% citric acid soln and enough  $\text{NH}_4\text{OH}$  to make soln alkaline to bromothymol blue. Add 5 ml of 10%  $(\text{NH}_4)_2\text{HPO}_4$  soln. Stir vigorously until a precipitate forms. Add 15 ml of  $\text{NH}_4\text{OH}$  and allow to stand at least 2 hours, stirring frequently. If only small quantities of Mg are present and no precipitate forms during stirring or after adding 15 ml of  $\text{NH}_4\text{OH}$ , allow to stand overnight. Transfer precipitate to a small filter or filtering crucible. Wash, and ignite slowly in crucible at temp. below  $900^\circ$  (preferably in muffle furnace with pyrometric control) until C is burned and then at ca  $1100^\circ$  for 1–2 hours. Cool, and weigh.

The residue consists of  $\text{Mg}_2\text{P}_2\text{O}_7$  and possibly  $\text{Mn}_2\text{P}_2\text{O}_7$  and  $\text{Ca}_3(\text{PO}_4)_2$ . If alcoholic filtrate is clear, the  $\text{Ca}_3(\text{PO}_4)_2$  will not exceed 0.3 mg and may be neglected. Correct for Mn as follows: Dissolve residue in 10 ml of  $\text{H}_2\text{SO}_4$  (1+9); transfer soln to 250 ml Erlenmeyer flask; and add 50 ml of  $\text{HNO}_3$  (1+3), 2 ml of sirupy  $\text{H}_3\text{PO}_4$  (sp. gr. 1.7), and 0.2 g of  $\text{KIO}_4$ . Boil 15–20 min., cool, and dilute to convenient volume. In another flask containing same amounts of reagents treated in similar way, match color by adding a standard  $\text{KMnO}_4$  soln, or compare with standard  $\text{KMnO}_4$  soln in colorimeter. From volume of  $\text{KMnO}_4$  soln required, or reading of colorimeter, calculate weight of  $\text{Mn}_2\text{P}_2\text{O}_7$  in residue. Subtract this weight from total weight, and regard difference as  $\text{Mg}_2\text{P}_2\text{O}_7$ , which contains 21.84% of Mg.



## 2.52

*Volumetric Method—Official*

Filter the precipitate of  $\text{MgNH}_4\text{PO}_4$  from 2.51 thru asbestos pad on Gooch crucible. Remove excess  $\text{NH}_3$  by washing with a soln of equal volumes of alcohol and  $\text{H}_2\text{O}$  (6–10 washings). Transfer pad and precipitate quantitatively to beaker with  $\text{H}_2\text{O}$ . To ca 50 ml add sufficient 0.1 *N*  $\text{H}_2\text{SO}_4$  from buret to dissolve precipitate, and use a small excess of the acid. Titrate excess acid with 0.1 *N*  $\text{NaOH}$ , using methyl orange as indicator. 1 ml of 0.1 *N* acid = 0.00122 g of Mg.

If Mn is present, add 1 ml of  $\text{H}_2\text{SO}_4$  to the soln from above titration, and transfer to 200 ml volumetric flask. Make to volume, mix, and pipet 50 ml of the clear soln into beaker. Add 5 ml of  $\text{H}_3\text{PO}_4$  and 0.3 g of  $\text{KIO}_4$  and heat 30 min., or until color development is complete. Dilute to measured volume containing not more than 20 p.p.m. of Mn and compare with a  $\text{KMnO}_4$  standard in a colorimeter.

Correct the previous titration, or calculated weight of Mg, for the Mn present, taking account of the dilutions.

## 2.53

**WATER-SOLUBLE MAGNESIUM (22)—TENTATIVE**

Weigh 1 g of sample into 500 ml volumetric flask, add 350 ml of  $\text{H}_2\text{O}$ , and boil for 1 hour. Cool, make to mark, mix and filter if necessary. Transfer to beaker, an aliquot containing less than 12 mg of Mg, usually 200 ml. Add ca 1 g of  $\text{NH}_4\text{Cl}$  for each 100 ml, a few drops of methyl red, and acidify with  $\text{HCl}$ . Proceed as directed in 2.51, line 3, beginning, "Partially neutralize with  $\text{NH}_4\text{OH}$ ," but add only 15 ml of saturated  $\text{NH}_4$  oxalate. After filtering off the Ca oxalate, add 2 ml of  $\text{HCl}$  to the filtrate and evaporate to 100 ml before adding citric acid and proceeding with the precipitation of  $\text{MgNH}_4\text{PO}_4$ .

## 2.54

**MAGNESIUM IN WATER-SOLUBLE COMPOUNDS (21)—OFFICIAL**

(Applicable to sulfate of potash magnesia, sulfate of magnesia, and kieserite)

Weigh 1 g sample into 250 ml volumetric flask, add 200 ml of  $\text{H}_2\text{O}$ , and boil for 30 min.; cool, and dilute to volume with  $\text{H}_2\text{O}$ . Proceed as directed in 2.51, beginning "Transfer an aliquot of the clear soln."

**ACID-SOLUBLE MANGANESE**

## 2.55

*Colorimetric Method (23)—Official*

(Applicable to samples with not more than 5% Mn)

Place 1 g of sample in 200 ml wide-necked volumetric flask or a 250 ml beaker. Add 10 ml of  $\text{H}_2\text{SO}_4$  and 30 ml of  $\text{HNO}_3$ . Heat gently until brown fumes diminish, then boil 30 min. If organic matter is not destroyed, cool, add 5 ml of  $\text{HNO}_3$ , and boil. Repeat this process until no organic matter remains, and boil until white fumes appear. Cool slightly, and add 50 ml of  $\text{H}_3\text{PO}_4$  soln (90 ml of  $\text{H}_2\text{O}$ , 10 ml of  $\text{H}_3\text{PO}_4$ ). Boil for a few minutes. Cool, make to 200 ml in a volumetric flask, mix, and let stand to allow precipitation of  $\text{CaSO}_4$ . Pipet 50 ml of clear soln into a beaker. Heat nearly to b.p., with stirring or whirling add 0.3 g of  $\text{KIO}_4$  for each 15 mg of Mn present, and proceed as directed in 27.59. At final dilution soln should contain not more than 200 p.p.m. of Mn. Calculate to Mn.

*Bismuthate Method (24)—Official*

## 2.56

**REAGENTS**

(a) *Sodium bismuthate powder*.—80%  $\text{NaBiO}_3$ , containing not more than 0.0005% of Mn, and not more than 0.002% of Cl.

(b) *Potassium permanganate soln.*—0.0910*N*. 2.876 g of  $\text{KMnO}_4$  in 1 liter of soln. 1 ml contains 1 mg of Mn. Standardize with Na oxalate.

(c) *Ferrous sulfate soln.*—0.091*N*. 25.3 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 25 ml of  $\text{H}_2\text{SO}_4$ , and 25 ml of  $\text{H}_3\text{PO}_4$  in 1 liter of soln. 1 ml = 1 mg of Mn. Standardize with  $\text{KMnO}_4$  near time of actual use. Place measured portion approximately equivalent to maximum quantity of Mn to be determined in Erlenmeyer flask containing 200 ml of cold  $\text{H}_2\text{SO}_4$  (3+97), and titrate with the  $\text{KMnO}_4$  soln.

## 2.57

## DETERMINATION

To 1 g of sample in Erlenmeyer flask (preferably 300 ml), add 5–10 ml of  $\text{HNO}_3$  and 7 ml of  $\text{H}_2\text{SO}_4$ . Evaporate on hot plate to white fumes. Add few drops of  $\text{HNO}_3$ , again evaporate to white fumes, and repeat until organic matter is destroyed. Cool. Add 100 ml of  $\text{H}_2\text{O}$ , 10 ml of  $\text{HNO}_3$ , and just enough  $\text{NaBiO}_3$  to give soln a strong permanganate color, or in case of small quantity of Mn, a slight excess of  $\text{NaBiO}_3$ . Boil gently 2–3 min. If the permanganate color or  $\text{MnO}_2$  disappears, cool somewhat, and repeat bismuthate treatment. (A permanent permanganate color or the persistence of  $\text{MnO}_2$  indicates a sufficient excess of bismuthate.) Add a saturated  $\text{NaHSO}_3$  soln dropwise while stirring until Mn compounds are reduced and soln clears. Avoid a large excess. Boil gently 2–3 min. Cool to room temp. and make to a volume of ca 100 ml. If soln contains less than 40 mg of Mn, proceed with determination. If more than 40 mg of Mn is present, transfer to 200 ml volumetric flask, add 5 ml of  $\text{H}_2\text{SO}_4$  and 10 ml of  $\text{HNO}_3$ , cool, dilute to volume, and mix. Pipet an aliquot containing not more than 40 mg of Mn into Erlenmeyer flask and dilute to 100 ml with  $\text{H}_2\text{O}$  soln containing 5 ml of  $\text{H}_2\text{SO}_4$  and 10 ml of  $\text{HNO}_3$  in 100 ml.

Before continuing, prepare suction filters of asbestos washed with the  $\text{H}_2\text{SO}_4$  and then with  $\text{H}_2\text{O}$ . (Glass filter tubes with perforated porcelain disks to support asbestos and connected with suction flask are satisfactory. Mn soln must not come in contact with rubber.) From this point complete determination without interruption. To the Mn soln at 20–30° add at least 0.25 g of  $\text{NaBiO}_3$  for each 10 mg of Mn. (It may be measured by weight or by volume of known approximate weight relationship.) Swirl contents of flask for 1 min., add 100 ml of  $\text{H}_2\text{O}$ , and mix. Filter with suction thru prepared filter and wash with cold  $\text{H}_2\text{SO}_4$  (3+97) until washings show no pink tint. Disconnect suction flask, and from buret add the  $\text{FeSO}_4$  soln until permanganate color disappears; then add at least 10% in excess with 1 ml as minimum excess. Titrate excess  $\text{FeSO}_4$  with the  $\text{KMnO}_4$  soln to faint pink. From the  $\text{KMnO}_4$  equivalent to the ml of  $\text{FeSO}_4$  soln used, subtract the  $\text{KMnO}_4$  used in back titration. From difference calculate percentage of Mn in sample.

## COPPER

*Long Volumetric Method (25)—Official*

## 2.58

## REAGENTS

(a) *Standard sodium thiosulfate soln.*—Dissolve 7.82 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

(b) *Standard copper nitrate soln.*—Place 2.000 g of pure Cu (electrolytic) in 1 liter volumetric flask, add 100 ml of  $\text{HNO}_3$ , heat until the Cu is dissolved, and dilute with  $\text{H}_2\text{O}$  to volume at room temp.

(c) *Potassium iodide soln.*—Dissolve 50 g of KI in enough  $\text{H}_2\text{O}$  to make 100 ml of soln.

(d) *Starch soln.*—Mix ca 1 g of soluble starch with enough cold  $\text{H}_2\text{O}$  to make a thin paste, add 100 ml of boiling  $\text{H}_2\text{O}$ , and boil while stirring for ca 1 min.

## 2.59

## DETERMINATION

Weigh 2 g of sample if less than 5% Cu. If more than 5% Cu, weigh sufficient quantity to furnish a little less than 0.1000 g of Cu. Place sample in 300 ml Erlenmeyer flask, and add 5–10 ml of  $\text{HNO}_3$  and 7.0 ml of  $\text{H}_2\text{SO}_4$ . Digest on hot plate to dense white fumes. If soln becomes dark due to organic matter, cool somewhat, add a little more  $\text{HNO}_3$ , and digest again to dense white fumes, repeating the operation if necessary until the organic matter appears to be destroyed. Cool, and add 25–30 ml of  $\text{H}_2\text{O}$ . Boil 1 min., remove from hot plate, and stir occasionally for ca 15 min. Filter into 250 ml Erlenmeyer flask and wash filter and residue 6 times with small portions of hot  $\text{H}_2\text{O}$ . Cool to room temp. and dilute to 100 ml.

Pass  $\text{H}_2\text{S}$  thru the soln in an Erlenmeyer flask for 10–15 min. Prepare a wash soln of 10 ml of  $\text{H}_2\text{SO}_4$ , plus enough  $\text{H}_2\text{O}$  to make 1 liter, and saturate with  $\text{H}_2\text{S}$ . Filter sample soln thru a paper of fine texture and wash paper and precipitate 7 times with small portions of the wash soln, keeping filter funnel covered with watch-glass as much of time as possible. Reserve filtrate for Zn determination.

Place paper and precipitate in glazed porcelain crucible and ignite at dull red heat until C is completely destroyed. Blow the  $\text{H}_2\text{S}$  gas out of precipitation flask and wash the  $\text{CuS}$  from the  $\text{H}_2\text{S}$  delivery tube into flask with Br water. Add 5 ml of  $\text{HNO}_3$  to the  $\text{CuO}$  in cold crucible and warm until the  $\text{CuO}$  is dissolved. (This may require 10 min., at end of which time insoluble specks may be disregarded.) Wash soln into precipitation flask with  $\text{H}_2\text{O}$  and dilute to 35 ml. For standardizing the  $\text{Na}_2\text{S}_2\text{O}_3$  soln add to another 250 ml Erlenmeyer flask an aliquot of the standard  $\text{Cu}(\text{NO}_3)_2$  soln and more  $\text{HNO}_3$ , so that an equivalent of 5 ml of  $\text{HNO}_3$  is present, and dilute to 35 ml. Hereafter treat all solns alike. Add an excess of Br water and a few glass beads. Boil until excess of bromine is entirely expelled and volume is less than 30 ml. Cool a little and add  $\text{NH}_4\text{OH}$  cautiously until mixture is distinctly alkaline. Boil until the odor of  $\text{NH}_3$  is very faint. Add 5 ml of acetic acid and boil a min. more. Cool to room temp. and dilute to 25–30 ml. Add 2 ml of the KI soln and titrate with the  $\text{Na}_2\text{S}_2\text{O}_3$  soln to a light yellow color. Add ca 1 ml of the cold starch soln and continue titration to disappearance of starch-iodine color. Calculate Cu equivalent of thiosulfate soln from titration of the soln containing the known amount of Cu and from this factor calculate amount of Cu in the sample soln.

## 2.60

*Short Volumetric Method (26)—Tentative*

Place 2 g of sample in 300 ml Erlenmeyer flask and add 10 ml of  $\text{HNO}_3$  and 5 ml of  $\text{H}_2\text{SO}_4$ . For standardizing the  $\text{Na}_2\text{S}_2\text{O}_3$  soln, 2.58(a), treat an aliquot of the standard  $\text{Cu}(\text{NO}_3)_2$  soln in the same manner as the sample. Digest on hot plate to white fumes. If soln becomes dark, due to organic matter, cool somewhat, add a little more  $\text{HNO}_3$ , and digest again to white fumes, repeating operation if necessary until organic matter appears to be destroyed. Cool, add 50 ml of  $\text{H}_2\text{O}$ , boil ca 1 min., and cool to room temp.

Add bromocresol green indicator soln, then  $\text{NH}_4\text{OH}$  until indicator changes to light green (pH 4). Cool again to room temp., and if the indicator changes back to a more acid color, add  $\text{NH}_4\text{OH}$  dropwise until indicator becomes light green again, avoiding an excess. Add 2 g of  $\text{NH}_4\text{HF}_2$ , mix well, and allow to stand ca 5 min. Add 8–10 g of KI, mix well, and titrate with  $\text{Na}_2\text{S}_2\text{O}_3$  soln to a light yellow color. Add ca 1 ml of the starch soln and continue titration slowly until color is nearly same as just before the addition of the KI and becomes no darker on standing 20 seconds. Report as % Cu.

## ZINC

## 2.61

*Gravimetric Method (27)—Tentative*

(Samples containing 0.1% or more of Zn)

For samples containing less than 0.20% of Zn weigh 10 g or just enough to furnish 4 mg of Zn; for samples containing 0.20% Zn or more, weigh 2 g. Treat samples as directed for the Cu method, 2.59, thru the separation of CuS. Evaporate combined filtrate and washings to ca 100 ml. If soln is darker than a light yellow or light green, add an excess of saturated  $\text{KMnO}_4$  soln and heat to boiling, adding more  $\text{KMnO}_4$  soln if necessary to maintain an excess. Now add 6% sulfurous acid soln until the Mn is reduced, and an excess of 1–2 ml, and continue evaporation to ca 80 ml. Cool, and add 5 ml of 40% citric acid soln and 2 drops of 0.4% bromophenol blue soln. Add  $\text{NH}_4\text{OH}$  to a slight change of the indicator color and cool to room temp. Adjust to pH 3.0 by adding  $\text{NH}_4\text{OH}$  or  $\text{H}_2\text{SO}_4$  (1+1) dropwise. (For comparison, place in another 250 ml Erlenmeyer flask 100 ml of 0.05% citric acid soln, pH 3.0, and add 2 drops of bromophenol blue.) Pass a rapid stream of  $\text{H}_2\text{S}$  thru sample soln for 45 min. Prepare wash soln containing 0.5 g of citric acid/liter and saturate with  $\text{H}_2\text{S}$ . Filter sample soln thru ashless paper of fine texture. Use a rubber policeman to loosen precipitate sticking to flask and delivery tube and wash onto filter with a jet of the prepared wash soln. Wash paper and precipitate 7 more times with small quantities of the wash soln, keeping funnel covered with watch-glass as much of time as possible. Place paper and precipitate in Pt crucible that has been ignited and weighed with cover. Ignite in uncovered crucible at a low temp., preferably in muffle, until paper is oxidized, then between 950 and 1000° for 1 hour. Place cover on crucible while hot, cool in desiccator containing  $\text{H}_2\text{SO}_4$ , and weigh as  $\text{ZnO}$ . Report as Zn.

## 2.62

*Colorimetric Method (26)—Tentative*

(For samples containing less than 4% of Zn)

To 2.5 g of sample in Kjeldahl flask add ca 10 ml of  $\text{HNO}_3$  and exactly 10 ml of  $\text{H}_2\text{SO}_4$ . Boil down to white fumes. If soln becomes dark due to organic matter add a little more  $\text{HNO}_3$  and boil down again to white fumes, repeating if necessary until organic matter is destroyed. Cool, and add 100 ml of  $\text{H}_2\text{O}$ . Boil 3–5 min. and cool to room temp. Filter with suction thru mat of filter paper pulp. Wash out flask and wash filter at least 5 times with the wash soln. Dilute filtrate to 250 ml with  $\text{H}_2\text{O}$  in volumetric flask. Dilute this soln to obtain a 10 ml aliquot that will contain ca 20 micrograms of Zn. Pipet such a 10 ml aliquot into a flask and titrate with normal  $\text{NH}_4\text{OH}$  until neutral to methyl red. Using another 10 ml aliquot, proceed as directed in 12.27, adding the same volume of the  $\text{NH}_4\text{OH}$  after adding the 40 ml of Soln A.

## 2.63

## FREE SULFUR (28)—TENTATIVE

Extract 1 g of sample with  $\text{CS}_2$  in a Soxhlet, allowing extraction thimble to drain at least 12 times. Transfer filtrate to a 250 ml beaker. Evaporate off the  $\text{CS}_2$  in a draft at room temp. Heat in drying oven at 60–70° for 20 min., then cool at room temp. Add 10 ml of a saturated soln of Br in  $\text{CCl}_4$ , cover, and allow to stand ca 30 min., stirring several times. Add 15 ml of  $\text{HNO}_3$ , cover, and allow to stand ca 30 min., stirring several times. Evaporate on hot plate to ca 5 ml. Add 20 ml of  $\text{HCl}$

and evaporate to ca 5 ml. Add ca 50 ml of  $H_2O$ , filter, and wash with 2% HCl. Add 2 drops of 0.4% bromophenol blue soln and then  $NH_4OH$  to first color change of the indicator. Add HCl, one drop at a time, until distinctly acid, then 5 drops in excess, dilute to 150 ml, heat to boiling, and add 10%  $BaCl_2 \cdot 2H_2O$  soln slowly dropwise until ca 50% excess is present. (1 ml of 10%  $BaCl_2 \cdot 2H_2O$  soln will precipitate ca 0.013 g of S.) Cover beaker and digest on steam bath at least 1 hour. Cool to room temp. and filter thru asbestos on a Gooch crucible that has been ignited at  $500^\circ$  and weighed. Wash 10 times with hot  $H_2O$ . Ignite in a muffle at  $500^\circ$  for at least 20 min. Cool in a desiccator and weigh as  $BaSO_4$ . Calculate as S.

#### ACID-FORMING OR NON-ACID-FORMING QUALITY (sg)—TENTATIVE

##### 2.64

##### REAGENTS

(a) *Mixed indicator*.—Weigh 0.1 g of bromocresol green and 0.02 g of methyl orange into agate mortar, triturate, and slowly add ca 2 ml of 0.1 N NaOH. Dilute to 100 ml with  $H_2O$ .

(b) *Sodium carbonate-sucrose soln*.—Dissolve 106 g of anhydrous  $Na_2CO_3$ , or 286 g of  $Na_2CO_3 \cdot 10H_2O$ , and 50 g of sucrose, in  $H_2O$ . Dilute to 1 liter. Pipet 10 ml into 250 ml Erlenmeyer flask, add 30 ml of normal HCl carefully, and boil gently few minutes to remove  $CO_2$ . Titrate with 0.5 N NaOH as directed below. The number of ml of 0.5 N NaOH used in titrating is the blank for the soln.

##### 2.65

##### DETERMINATION

If the fertilizer mixture, ground as directed under 2.2, contains less than 30% as the sum of percentages of total N, available  $P_2O_5$ , and water-soluble potash, weigh 1 g of mixture into 100 or 150 ml porcelain or Pyrex glass beaker. If the sum of these percentages is 30 or more, use 0.5 g, and for salts of Na or K use 0.25 g. With a pipet or buret add 10 ml of the  $Na_2CO_3$ -sucrose soln, and mix thoroly with the fertilizer, except for unmixed nitrate salts or for mixed fertilizers containing considerable nitrate N. For these, substitute 0.25 g of carbon black for the sucrose. Place in sand bath to depth of mixture in beaker and evaporate to complete dryness. (A cone of ashless filter paper folded so that the base will just slip into the beaker and touch the sides all around, with apex cut off to form a vent ca 3 mm in diam., may be used to avoid loss by spattering.) Place beakers in furnace heated to ca  $250^\circ$ , and raise temp. gradually to  $575$ – $600^\circ$  (dull red). Hold at this temp. 1 hour. (It is not necessary that all carbon be removed.) Remove beaker and allow to cool. Add 50 ml of  $H_2O$ , cover with watch-glass, and add 30 ml of normal HCl thru lip of beaker. After effervescence ceases, place covered beaker on hot plate or steam bath and maintain just below boiling point 1 hour. Titrate by one of the following methods:

(a) *With mixed indicator*.—Filter soln thru disk of paper, or pad of asbestos that has been digested with normal HCl and washed free from acid with  $H_2O$ , using Gooch crucible and suction. Wash with hot  $H_2O$ . To clear filtrate (ca 100 ml) add 0.4 ml of the mixed indicator, and titrate to light green color (pH 4.3) until green definitely predominates over yellow. (Duplicate soln of fertilizer ash displaying maximum acid color for this indicator may be used as comparison to determine first change. Titration is conveniently carried out on white porcelain plate, using artificial daylight bulb placed at convenient angle above and back of plate.)

(b) *With glass electrode*.—Cool to room temp. and without filtering titrate soln to pH 4.3 in 150 ml beaker with 0.5 N NaOH, using glass electrode apparatus or other standard means of electrometric titration and a continuous stirrer. Make usual blank titration, using the glass electrode.

Subtract algebraically ml of 0.5 *N* NaOH used in titrations from the blank, 2.64(b). For 1 g sample multiply result by 50; 0.5 g sample, by 100; 0.25 g sample, by 200. Positive values represent excess base in ash in pounds of CaCO<sub>3</sub> equivalent per ton of fertilizer. Negative values represent excess acidity in same terms.

Percentage of N found (2.27 or 2.28)  $\times 35.7$  is considered the acid-forming power of the N in terms of pounds of CaCO<sub>3</sub> equivalent per ton of fertilizer, and is given a negative sign in calculating net acid-base balance.

Percentage of citrate-insoluble P<sub>2</sub>O<sub>5</sub> (2.16)  $\times 28.2$  = alkalinity equivalent to 2 of the 3 Ca atoms of Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>, expressed as pounds of CaCO<sub>3</sub> equivalent per ton of fertilizer. Correct net balance for fertilizer for this basicity, assumed to be relatively inactive in the soil, by giving the value a negative sign.

The algebraic sum of the acid-base balance of the ash and the corrections for N and citrate-insoluble P<sub>2</sub>O<sub>5</sub> is the net balance of the fertilizer as pounds of CaCO<sub>3</sub> equivalent per ton. If negative, the fertilizer is considered acid forming; if positive, it is considered non-acid forming.

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- (24) Ibid., 24, 268 (1941).
- (25) Ibid., 305.
- (26) Ibid., 25, 77, 78, 352 (1942).
- (27) Ibid., 77, 361.
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### 3: AGRICULTURAL LIMING MATERIALS (1)

#### 3.1

##### DIRECTIONS FOR SAMPLING—TENTATIVE

Take sample representative of lot or shipment that does not contain disproportionate quantity of the surface or of any modified or damaged zone, in following manner:

(a) *Burnt, or lump lime, in bulk.*—Collect composite sample of not less than 10 shovelfuls/car, with proportionate quantities from smaller lots, taking each shovelful from different part of lot or shipment. Crush immediately to pass circular opening 2" in diam., mix thoroly and rapidly, quarter down to 5 lb. sample, and place in properly labeled, dry, air-tight container.

(b) *Burnt, or lump lime, in barrels.*—Select at random 5 barrels from each lot or shipment of 20 tons or less and 1 additional barrel for each additional 5 tons. Take not less than 10 lbs. from each barrel selected and treat as directed under (a).

(c) *Hydrated lime and ground burnt lime, in bags.*—Select 10 bags from different parts of each lot or shipment of 20 tons or less and 1 additional bag for each additional 5 tons. From each of bags sampled withdraw core from top to bottom by means of sampling tube, mix these portions thoroly and rapidly on heavy, sized paper or oilcloth, quarter down to 2 lb. sample, and place in properly labeled, dry, air-tight container.

(d) *Ground limestone and ground marl, in bags.*—Proceed as directed under (c).

(e) *Ground limestone, ground burnt lime, and ground marl, in bulk.*—By means of slotted sampling tube, withdraw samples to full sampler depth from 10 points in lot or shipment, and proceed as directed under (c), beginning "mix these portions."

#### 3.2

##### PREPARATION OF SAMPLE—TENTATIVE

Grind sample in porcelain mortar or porcelain ball mill to pass 60-mesh sieve, mix thoroly, and preserve in air-tight container.

##### NEUTRALIZATION VALUE—TENTATIVE

#### 3.3

##### REAGENTS

(a) *Sodium hydroxide soln.*—0.25 *N*. Prepare free from carbonates and store in Pyrex bottle provided with siphon tube and with guard tubes containing soda-lime, or other suitable device, to prevent absorption of CO<sub>2</sub> from air.

(b) *Nitric acid.*—0.5 *N*. Standardize against (a), using phenolphthalein indicator, 2.10(d).

#### 3.4

##### DETERMINATION

Place 0.5 g of burnt or hydrated lime (1 g of ground limestone or ground marl), prepared as directed under 3.2, in 250 ml Erlenmeyer flask; add 50 ml of the HNO<sub>3</sub> and boil gently 5 min. Cool, and titrate excess of acid with the NaOH soln, using phenolphthalein indicator. Report as percentage of CaO in burnt and hydrated lime and as percentage of CaCO<sub>3</sub> equivalent for limestone and marl.

##### CAUSTIC VALUE (§)—OFFICIAL

#### 3.5

##### APPARATUS

In illustration (Fig. 6), *A* is 500 ml Erlenmeyer flask of Pyrex glass, and *F* is filter cone packed nearly full with cotton, which is covered to depth of 2–3 mm

with lightly compacted, macerated filter paper. (A fritted glass filter may be substituted for the home-made filter shown in Fig. 6.) Filter is connected with siphon tube *B* by means of thick-walled rubber tubing. Receiving flasks *m* and *n* are calibrated to deliver 50 and 100 ml, respectively. *S* is suction flask.

## 3.6

## DETERMINATION

Transfer portion of sample, 3.2, to weighing bottle and determine weight of bottle and contents in an atmosphere of minimum moisture and  $\text{CO}_2$  content. By means of polished, narrow-pointed spatula calibrated to hold ca 1.5 g, withdraw charge to be used and determine its exact weight by difference. Introduce charge directly into dry flask (*A*), provided with tightly fitting rubber stopper.

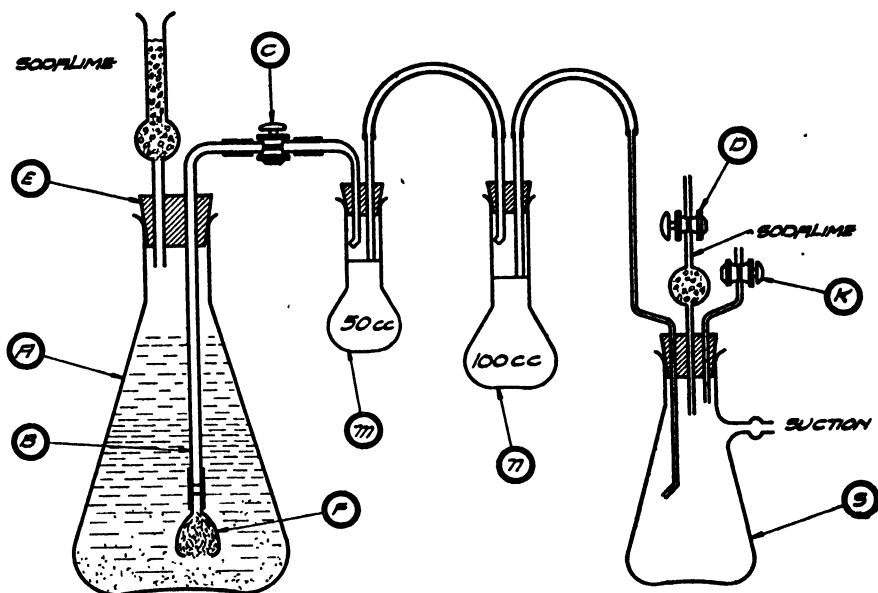


FIG. 6.—APPARATUS FOR AUTOMATIC FILTRATION AND MEASUREMENT OF LIME SOLUTIONS

Prepare a sugar soln *immediately before use* by placing 25 g of granulated sugar in measuring flask calibrated to deliver 500 ml. Dissolve sugar with cold  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  and make to mark. Holding both Erlenmeyer flask containing charge and flask containing sugar soln in a slightly inclined position, insert neck of sugar soln flask short distance into Erlenmeyer flask, and carefully transfer sugar soln while simultaneously and synchronously agitating both flasks by rotary motion to prevent granulation of lime. Stopper Erlenmeyer flask securely; agitate; and add, if desired, quantity of clean dry beads. Effect complete soln of uncoated caustic lime by six 1 min. agitations at intervals of 2 or 3 min. Crush any undisintegrated particles of sample by careful twisting of stopper after inverting flask to trap them in space between stopper and neck of flask. Allow 15 min. further contact between lime and sugar soln, and filter as follows:

Connect filter cone *F* with siphon *B* and close stopcock *D*. Connect receiving flasks, apply suction, and quickly connect Erlenmeyer flask (*A*) containing lime



soln with stopper *E*. Open stopcock *C* and filter 25–50 ml of soln. Close *C* and open *D* to release suction. Remove *m* and replace with another dry flask of same kind. Close *D*, open *C*, and continue filtration until both *m* and *n* have been filled at least to the marks. To disconnect system, close stopcock *C* and press outlet of flask *m* down gently and then outlet of flask *n* to remove any excess of liquid above the marks. Permit intermediate connection to empty, open stopcock *D*, and remove *m* and *n*. Titrate first 50 ml, or pilot aliquot, of filtered soln with 0.5 *N* HCl, using phenolphthalein indicator. Run twice the volume of 0.5 *N* acid required for this titration into covered 200 ml beaker; add second, or 100 ml aliquot, of filtered soln to this acid and phenolphthalein indicator; and complete titration.

Calculate caustic value of sample by the formula:

$X = \frac{7A}{W}$ , in which *X* = percentage of active CaO; *A* = ml of 0.5 *N* acid used/100 ml of lime soln; and *W* = weight of charge.

## 3.7

## CARBON DIOXIDE—TENTATIVE

Proceed as directed under 1.6, using 5 g of burnt or hydrated lime (1 g of ground limestone or ground marl), prepared as directed under 3.2. Report as percentage of CaCO<sub>3</sub>.

## 3.8

## TOTAL CALCIUM OXIDE—TENTATIVE

Place 1 g of burnt or hydrated lime (2 g of ground limestone or ground marl), prepared as directed under 3.2, in hard glass beaker of 250 ml capacity; add 25 ml of H<sub>2</sub>O, 10 ml of HCl, and few drops of HNO<sub>3</sub>; boil 10 min.; and evaporate to dryness. Separate and remove insoluble matter, SiO<sub>2</sub>, and Fe and Al oxides, as directed under 1.12 and 1.13. Determine CaO as directed under 1.14.

## 3.9

## TOTAL MAGNESIUM OXIDE—TENTATIVE

Proceed as directed under 1.16, using combined filtrate and washings from CaO determination, 3.8.

## 3.10

## MECHANICAL ANALYSIS OF GROUND LIMESTONE—TENTATIVE

Transfer 100 g of original material to set of 10-, 20-, 40-, 60-, 80-, and 100-mesh standardized sieves that comply with specifications of Bureau of Standards. Sift, shaking 5 min. on the 80- and 100-mesh sieves and breaking lumps by means of a soft rubber pestle if the material has a tendency to cake. Weigh material retained on each sieve and that passing 100-mesh sieve and report as percentages of total weight.

## 3.11

NEUTRALIZATION VALUE OF CALCIUM SILICATE SLAGS (*s*)—TENTATIVE

(a) *Blast furnace slags*.—Weigh 0.5 g charge ground to pass 80-mesh sieve and transfer into 250 ml Erlenmeyer flask. Wash down with small portions of H<sub>2</sub>O and introduce 35 ml of 0.5 *N* HCl while whirling. Heat to gentle boil over Bunsen burner, *agitating the suspension continuously* until bulk of sample has dissolved. Maintain boiling for 5 min., cool to room temp., transfer to 150 ml beaker, and dilute to ca 80 ml. Titrate with 0.5 *N* NaOH to pH 4.8 as determined by a glass electrode. Net ml of acid used  $\times 5$  = neutralization value of the slag in terms of percentage CaCO<sub>3</sub> equivalence.

(b) *Slags from rock phosphate reduction furnaces*.—Weigh 0.5 g charge and transfer to 250 ml beaker. Wash down with small portions of H<sub>2</sub>O and introduce 50 ml

of acetic acid (1+4). Stir suspension continuously during addition of acid. Heat to boiling 5 min., stirring frequently. Evaporate to dryness on steam bath. Add 20 ml of acetic acid (1+4), dilute to 150 ml, and heat to boiling; add  $\text{NH}_4\text{OH}$  (1+1) to the distinct yellow of methyl red. Digest ca 10 min. on hot plate. Filter by gravity on 9 cm paper, catching filtrate in 100×50 mm lipped Pyrex crystallizing dish; wash beaker 3 times and the filter 5 additional times with neutral 0.5 *N*  $\text{NH}_4$  acetate. Evaporate filtrate on hot plate. Prevent spattering by adjustment of heat so that the bubbles that break thru the viscous surface film are released gently. (Dehydration may be expedited by 2 or 3 repeated treatments with 25 ml of hot  $\text{H}_2\text{O}$  and evaporation.) Continue heating residue on hot plate until odor of acetic acid cannot be detected. Heat an additional 10 min. at full heat of hot plate and then ignite 10 min. in electric furnace at 550°. Cool, wet residue with 15 ml of  $\text{H}_2\text{O}$ , place cover-glass over dish, and introduce 25 ml of 0.5 *N*  $\text{HCl}$  thru lip of dish. Heat 5 min. over Bunsen burner to gentle simmer. Rinse cover-glass; filter suspended matter on 9 cm filter, catching filtrate in 250 ml Erlenmeyer flask, and wash dish and filter 3 times with hot  $\text{H}_2\text{O}$ . Titrate excess acid with 0.5 *N*  $\text{NaOH}$  to the distinct yellow of methyl red. Net acid used × 5 = neutralization value of slag in terms of percentage  $\text{CaCO}_3$  equivalence.

#### SELECTED REFERENCES

- (1) J. Assoc. Official Agr. Chem., 7, 252 (1924); U. S. Bur. Standards Circs. 96, 118, 143, 150, 153; Am. Soc. Test. Materials, Tentative Standards, 1923, p. 277.
- (2) Ind. Eng. Chem., 20, 312 (1928); J. Assoc. Official Agr. Chem., 11, 153 (1928); 14, 283 (1931).
- (3) J. Assoc. Official Agr. Chem., 27, 74, 532 (1944); 28, 310 (1945).

## 4. COSMETICS

### DEODORANTS AND ANTI-PERSPIRANTS

#### ALUMINUM AND ZINC—TENTATIVE

##### 4.1

##### REAGENTS

(a) *8-Hydroxyquinoline soln.*—Dissolve 5 g of 8-hydroxyquinoline in 12 ml of acetic acid, dilute to 100 ml with  $H_2O$ , and filter if not clear. Prepare fresh soln at least every 2 weeks.

(b) *Ammonium acetate soln.*—Approximately 2 *N*. Dissolve 150–160 g of  $NH_4$  acetate in 1 liter of  $H_2O$  and filter if not clear.

(c) *Hydrochloric acid.*—Approximately 2 *N*.

(d) *Ammonium hydroxide.*—Approximately 2 *N*. (Amount of  $NH_4OH$  required to neutralize 20 ml of the 2 *N*  $HCl$  should be known to within  $\pm 2$  ml.)

##### 4.2

##### PREPARATION OF SAMPLE

(a) *Liquids.*—Dilute 5 ml of sample to 250 ml with  $H_2O$  in volumetric flask (If perfume oils separate, filter before taking aliquot for analysis.)

(b) *Creams and pastes.*—Weigh accurately 2–3 g of sample into 200 ml beaker. Add 5 ml of  $HCl$  and ca 50 ml of  $H_2O$ , and heat until oils liquefy and separate; cool until oils solidify, and decant aqueous layer thru fluted filter into 250 ml volumetric flask. Return filter to original beaker and macerate thoroly. Repeat above extraction twice, decant as before, and finally wash residue and paper thoroly with  $H_2O$ . (It is not necessary to return filter paper to beaker after these extractions.) Cool combined extracts to room temp., dilute to mark with  $H_2O$ , and mix.

(c) *Solids.*—Weigh accurately 2–3 g of material into 200 ml beaker, add 5 ml of  $HCl$  and ca 50 ml of  $H_2O$ , and heat to boiling. Cool, and filter thru fluted filter into 250 ml volumetric flask. (If filtrate is cloudy, refilter thru fine quantitative filter paper.) Wash beaker and filter paper thoroly with  $H_2O$ . Cool flask and contents to room temp., dilute to mark with  $H_2O$ , and mix.

##### 4.3

##### DETERMINATION

(a) *Interfering metals absent.*—Take aliquot of sample soln containing 12–25 mg of Al or 20–60 mg of Zn. Add 1–2 drops of phenolphthalein indicator, 2.10(d), and then add the 2 *N*  $NH_4OH$  until neutral or until faint permanent turbidity results. Add 5 ml of acetic acid (1+9), dilute to ca 100 ml, and heat to 70–90°. Add 10 ml of the 8-hydroxyquinoline soln and then slowly add the  $NH_4$  acetate soln until 20 ml (see note) in excess of amount required to produce a permanent precipitate has been added. (If permanent precipitate forms on addition of the 8-hydroxyquinoline, add 20 ml of the  $NH_4$  acetate soln.) Heat below b.p. for 2–5 min. and set aside for 30–60 min. (Moderate excess of 8-hydroxyquinoline is required for complete precipitation; if sufficient reagent has been added, soln will be yellow at this point; if it is not, repeat precipitation, using larger amount of 8-hydroxyquinoline.) Filter thru tared Gooch crucible, wash thoroly with  $H_2O$ , dry at 130–140° for 1–2 hours, cool, and weigh. Dry again for 30 min., cool, and weigh. Repeat until wt. is constant ( $\pm 0.3$  mg). (Alternatively, precipitate may be dried overnight.)

Wt. of precipitate  $\times 0.05872 = Al$ .

Wt. of precipitate  $\times 0.1849 = Zn$ .

NOTE: Final pH of soln from which the metals are precipitated should be 4.9–5.1. Amount of  $\text{NH}_4$  acetate soln required to produce this pH should be determined experimentally each time a new set of reagents is prepared. If the  $\text{NH}_4$  acetate used is of usual purity, ca 20 ml of the soln will be required.

(b) *In presence of magnesium.*—Precipitate as directed under (a) and set aside for ca 30 min. Decant most of liquid thru rapid quantitative filter paper (part or all of precipitate may be transferred to paper if necessary) and discard filtrate. Place beaker in which precipitation was made under funnel and dissolve precipitate on paper in hot 2 *N* HCl (20 ml of acid is usually sufficient if added in several small portions). Wash paper and funnel with 20–30 ml of  $\text{H}_2\text{O}$ . Add 2 ml of the 8-hydroxyquinoline soln, 5 ml of acetic acid (1+9), and an amount of 2 *N*  $\text{NH}_4\text{OH}$  equivalent to the 2 *N* HCl used to dissolve the precipitate (do not use an excess). Dilute to ca 100 ml, heat to 70–90°, and continue as directed in (a), beginning “slowly add the  $\text{NH}_4$  acetate.”

## DEPILATORIES

### SULFIDES IN POWDERS (1)—OFFICIAL

#### 4.4

##### REAGENTS

(a) *Arsenious acid soln.*—0.1 *N*. Dissolve 4.9470 g of  $\text{As}_2\text{O}_3$  in 40 ml of *N* NaOH. Add 40 ml of *N*  $\text{H}_2\text{SO}_4$  and make to 1 liter. (Soln should be neutral to litmus.)

(b) *Standard iodine soln.*—0.1 *N*. Standardize against the 0.1 *N*  $\text{As}_2\text{O}_3$  soln.

#### 4.5

##### DETERMINATION

Pipet exactly 50 ml of the  $\text{As}_2\text{O}_3$  soln into 250 ml glass-stoppered volumetric flask. Weigh sample of the depilatory containing less than 0.12 g of sulfide calculated as  $\text{H}_2\text{S}$  and transfer to flask, washing down any material adhering to sides of flask with  $\text{H}_2\text{O}$ . Add 20 ml of HCl (1+1), stopper immediately, and shake vigorously until sample is decomposed. (If sample contains  $\text{CaCO}_3$ , add the 20 ml of acid slowly thru dropping funnel fitted with rubber stopper to fit 250 ml volumetric flask. Shake gently, allowing liberated  $\text{CO}_2$  to bubble up thru the acid. When reaction has subsided drain remainder of acid into flask, remove funnel, stopper flask, and shake vigorously.) Cool to room temp. and dilute to volume with  $\text{H}_2\text{O}$ . Filter thru dry filter into dry flask. Withdraw 100 ml of filtrate into 300 ml Erlenmeyer flask, add 5 ml of starch soln and sufficient of the I soln to form blue color. Make alkaline with  $\text{NaHCO}_3$ , adding 1–2 g in excess. Titrate to permanent blue color with the I soln.

1 ml of 0.1 *N*  $\text{As}_2\text{O}_3$  = 0.005411 g of CaS or 0.01271 g of BaS.

## HAIR PREPARATIONS

### 2,5-DIAMINOTOLUENE IN HAIR DYES AND RINSES

#### *Acetylation Method (2)—Official, First Action*

#### 4.6

##### REAGENTS

(a) *Sodium hydroxide soln.*—(1+1). Dissolve 100 g of reagent-grade NaOH in 100 ml of  $\text{H}_2\text{O}$ . Allow to stand until clear.

(b) *Acetic anhydride.*—U.S.P., less than 0.003% of non-volatile material.

## 4.7

## DETERMINATION

Place 5–20 ml aliquot of amine soln containing 0.05–0.15 g of the diamine in small separator. Add ca 0.05 g of  $\text{Na}_2\text{SO}_3$  and a volume of the  $\text{NaOH}$  (1+1) equal to 55% of the previously measured volume of sample soln. (It is convenient to add the  $\text{NaOH}$  from Mohr pipet with wide tip.) Cool contents of separator as rapidly as possible, add 20 ml of ether, and shake gently for ca 30 seconds. Let stand ca 1 min. and draw off  $\text{NaOH}$  layer into second separator. Be sure to remove all aqueous layer. (It does no harm to transfer 1–2 ml of ether layer also.) Carefully decant ether layer thru pledget of cotton placed in long-stemmed funnel into tared evaporating dish in such a way that none of aqueous layer that drains from sides of funnel is transferred with the ether. Wash first separator with 20 ml of ether and drain ether into separator containing  $\text{NaOH}$  layer. Make a second extraction as before, return aqueous layer to first separator, and decant ether layer thru the cotton pledget into evaporating dish. Continue in this manner until five extractions have been made. Wash the funnel and cotton with a little ether and evaporate combined extracts on steam bath to ca 10 ml. Add 1 ml of acetic anhydride and continue evaporation to dryness. Add few ml of alcohol and evaporate until odor of acetic anhydride disappears. Dry residue at  $100^\circ$  for 15 min., cool, and weigh the diacetyl derivative of the diamine. Repeat drying until weight is constant ( $\pm 0.5$  mg).

$$\text{Diacetyl 2,5-diaminotoluene} \times 0.592 = \text{2,5-diaminotoluene.}$$

Check purity of diacetyl derivative by determining its melting point. The m.p. of diacetyl 2,5-diaminotoluene is  $219\text{--}220^\circ$ .

*Dichlorimide Method (2)—Official, First Action*

## 4.8

## REAGENTS

(a) *Sodium hypochlorite soln.*—5% U.S.P. soln of  $\text{NaOCl}$ .

(b) *Sodium arsenite soln.*—10%. Dissolve 10 g of  $\text{Na}$  arsenite in 100 ml of  $\text{H}_2\text{O}$ ; or dissolve 8.5 g of  $\text{As}_2\text{O}_3$  and 15 g of  $\text{NaOH}$  in 100 ml of  $\text{H}_2\text{O}$ , heating to obtain soln.

## 4.9

## DETERMINATION

To separator containing 5 ml of the 5%  $\text{NaOCl}$  soln and ca 1 g of  $\text{NaHCO}_3$  add, by means of pipet or buret, an aliquot of a soln of sample representing 0.01–0.08 g of diamine. If insufficient  $\text{NaOCl}$  is indicated by presence of brown color while soln is being added, repeat operation, using more  $\text{NaOCl}$  or smaller aliquot. Thoroughly mix the soln during addition of sample aliquot by gently swirling separator. After charge has been added, stopper separator and shake for ca 10 seconds. Add 10 ml of the  $\text{Na}$  arsenite soln, stopper separator, and shake again. Extract the dichlorimide with two successive 25 ml portions of  $\text{CHCl}_3$  and combine extracts in second separator. Wash combined extracts with 10 ml of  $\text{H}_2\text{O}$  and filter thru pledget of cotton into an I flask. Make additional extraction, wash with the  $\text{H}_2\text{O}$ , and combine with major portion. Add 50 ml of  $\text{H}_2\text{O}$  containing 1 g of  $\text{KI}$  and 3 ml of  $\text{HCl}$  to combined  $\text{CHCl}_3$  extracts, stopper flask, and shake vigorously for 1 min. Titrate liberated I with 0.1  $N$   $\text{Na}_2\text{S}_2\text{O}_4$ . Stopper flask and shake vigorously at intervals during titration. (The I in the  $\text{CHCl}_3$  acts as indicator.) Toward end of titration add starch soln for final end point. Each ml of 0.1  $N$   $\text{Na}_2\text{S}_2\text{O}_4$  = 0.002035 g of 2,5-diaminotoluene.

## PARAPHENYLENEDIAMINE IN HAIR DYES AND RINSES—OFFICIAL

## 4.10

*Acetylation Method (3)—Official*

## APPARATUS

*Continuous extraction apparatus.*—Any continuous extractor of appropriate design (Fig. 56, 33.17; Fig. 65, 39.112).

## 4.11

## PREPARATION OF SAMPLE

(a) *Powders or dry mixtures.*—Put 1–2 g of the powder directly into 50 ml volumetric flask, add 2 ml of HCl (1+1), and make to volume with H<sub>2</sub>O.

(b) *Aqueous preparations.*—Dilute if necessary so that 5 ml aliquot will contain 0.1–0.3 g of p-phenylenediamine.

## 4.12

## DETERMINATION

Pipet 5 ml aliquot of prepared soln into extractor and add sufficient anhydrous Na<sub>2</sub>CO<sub>3</sub> to render aqueous layer alkaline to litmus. Completely extract with CHCl<sub>3</sub>, remove flask, and transfer CHCl<sub>3</sub> soln to 100 ml beaker, rinsing flask with few small portions of CHCl<sub>3</sub>. Evaporate CHCl<sub>3</sub> to ca 25 ml volume and add slowly with stirring 1 ml of acetic anhydride. Allow to stand 1 hour and filter on weighed Gooch crucible. Wash beaker and precipitate with three or four 5 ml portions of CHCl<sub>3</sub>. Use great care in removing last traces of precipitate from beaker. Dry to constant weight at 120° and weigh precipitate of diacetyl p-phenylenediamine, C<sub>8</sub>H<sub>8</sub>(NHCOCH<sub>3</sub>)<sub>2</sub> (1:4). Diacetyl derivative  $\times 0.5626$  = p-phenylenediamine.

Check purity of diacetyl derivative by determination of its melting point. M.p. of diacetyl p-phenylenediamine is 312–314°.

## 4.13

*Dichlorimide Method (Benzoquinone Method) (4)—Official*

Proceed as directed in 4.9. 1 ml of 0.1 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> = 0.001801 g of p-phenylenediamine.

## 4.14

## SALICYLIC ACID IN HAIR LOTIONS (5)—OFFICIAL

Acidify 25 ml of sample in 250 ml beaker with 2 ml of 10% HCl. Dealcologize by heating at not more than 70°, if possible by use of current of air at room temp. Transfer to separator and dilute with H<sub>2</sub>O to ca 25 ml. Since CHCl<sub>3</sub> is to be used later as extracting solvent, make transfer by washing from beaker to separator with 2 or 3 portions of CHCl<sub>3</sub>, totaling 25 ml, repeating washing with H<sub>2</sub>O in portions totaling 25 ml, thus obtaining indicated aqueous dilution.

Extract with four 25 ml portions of CHCl<sub>3</sub> (includes 25 ml used in transfer of sample to separator). Wash each CHCl<sub>3</sub> extract with 5 ml of H<sub>2</sub>O and filter into 150 ml beaker thru CHCl<sub>3</sub>-saturated pledget of cotton. Wash the 5 ml of H<sub>2</sub>O with CHCl<sub>3</sub>, filtering these washings into same beaker. Evaporate the CHCl<sub>3</sub> on steam bath to volume of 20–25 ml, allowing this amount to evaporate spontaneously to volume of 5 ml.

Transfer remaining 5 ml to separator, with enough CHCl<sub>3</sub> as rinsing agent to make volume of ca 30 ml in separator. Extract with three 5 ml portions of 5% NaHCO<sub>3</sub> soln and one 5 ml portion of H<sub>2</sub>O. Wash combined extracts with a 10 ml portion of CHCl<sub>3</sub> and discard the CHCl<sub>3</sub>.

Filter combined NaHCO<sub>3</sub> solns thru filter paper into 100 ml volumetric flask.

Rinse separators and wash filter with  $H_2O$  until volume of filtrate reaches 100 ml mark. Agitate flask to obtain uniformity of soln.

Proceed as directed in 39.29, line 2, beginning, "Transfer aliquot of this soln." Before adding the 0.1 *N* bromide-bromate soln, carefully neutralize aliquot in iodine flask to liberate  $CO_2$  from the bicarbonate present, then make alkaline with one drop of 10% NaOH soln and continue as directed. 1 ml of 0.1 *N* KBr-KBrO<sub>3</sub> soln = 0.00230 g of salicylic acid.

#### SELECTED REFERENCES

- (1) J. Assoc. Official Agr. Chem., 23, 440 (1940); 25, 113 (1942); 27, 112 (1944).
- (2) Ibid., 22, 159 (1939); 26, 117 (1943); 28, 86 (1945).
- (3) Ibid., 23, 717 (1940); 27, 112 (1944).
- (4) Ibid., 22, 159 (1939); 25, 113 (1942); 27, 112 (1944).
- (5) Ibid., 25, 112 (1942); 26, 355 (1943); 27, 112 (1944).

## 5. ENZYMES

### PROTEOLYTIC ACTIVITY OF PAPAIN (1)—TENTATIVE

#### 5.1

##### PREPARATION OF SAMPLE

(a) *Unactivated*.—If the enzyme preparation is a solid, finely divide it by grinding to smooth paste in small mortar with a little freshly boiled cold  $H_2O$ . Then suspend the enzyme in cold boiled  $H_2O$  in proportion of 10 mg of original preparation/ml. After 5–10 min. centrifuge suspension and discard sediment.

(b) *Activated*.—Proceed as directed in (a), but use half-saturated  $H_2S$  water instead of boiled water. After centrifuging, incubate the enzyme soln at  $40^\circ$  for 1 hour to complete activation.

#### 5.2

##### REAGENTS

(a) *Casein soln*.—Make a 6% soln of Hammarsten's casein by rubbing up 60 g with a little  $H_2O$  in mortar and gradually adding 60 ml of 1 *N* NaOH and  $H_2O$  until volume totals 1 liter. Heat the viscous soln for 30 min. in bath of boiling  $H_2O$ , cool, and filter (glass wool) if necessary.

(b) *Buffer soln*.—Prepare 0.2 *M* monosodium citrate soln by partial neutralization of citric acid with NaOH.

(c) *Titrating soln*.—0.1 *N* alcoholic KOH.

(d) *Indicator*.—1% alcoholic soln of thymolphthalein.

#### 5.3

##### DETERMINATION

Place 10 ml of the casein soln and a small charge of 4 mm diam. glass beads in each of several 125 ml glass-stoppered bottles, and bring bottles and contents to  $40^\circ$ . Add desired volume of the prepared enzyme soln, but do not use more than 4 ml. If this quantity is insufficient (see later), prepare a more concentrated soln of the enzyme. Add immediately exactly 3 ml of the buffer soln (pH of system should then be  $5.0 \pm 0.1$ ). Shake bottle vigorously for a few seconds and place in constant temp. water bath at  $40^\circ$ .

Incubate mixture for 20 min. at  $40^\circ$ , counting time from addition of buffer. Add 1 ml of the indicator and begin titrating with the titrating soln. As soon as a deep blue color appears, shake bottle until color is discharged or precipitate is completely dissolved. (It is usually best to add the alkali in doses of ca 0.5 ml at a time.) When all precipitated casein has been brought into soln, transfer contents of bottle to 400–500 ml flask and rinse out bottle 2 or 3 times with alcohol, using a total of 25 ml for this purpose. Add sufficient of the alcoholic KOH to restore the blue color in the titration, then add 175 ml of boiling alcohol. Carefully add more alcoholic KOH until a pale but distinct blue color persists in soln.

Make a control titration exactly as described, but do it immediately after the addition of the buffer and therefore without any incubation time. Difference between the titration of the undigested sample and that of the digested sample is a measure of the proteolytic activation of the enzyme.

#### 5.4

##### CALCULATION OF PROTEINASE UNIT

For smaller quantities of enzyme the extent of hydrolysis determined by the titration described is a straight line function of the amounts of papain used. For accurate work determine this straight line by making several titrations with differ-



ent quantities of enzyme. If the quantities of papain used are too large, the straight-line relationship will no longer hold; if they are too small, the determination will be inaccurate. Quantities of enzymes giving titration differences of 0.6–1.2 ml of 0.1 *N* KOH are recommended.

The unit of papain may be considered the quantity of enzyme that produces, under the conditions outlined, a titration difference of 1 ml of 0.1 *N* KOH, determined either graphically or arithmetically. The value of the original preparation is then expressed in units/mg, or as mg of the papain preparation necessary to make one unit.

#### SELECTED REFERENCE

- (1) J. Assoc. Official Agr. Chem., **18**, 140 (1935); **19**, 373 (1936); **21**, 97 (1938).

## 6. INSECTICIDES AND FUNGICIDES

### GENERAL METHODS

#### 6.1

##### PREPARATION OF SAMPLE—OFFICIAL

Thoroughly mix all samples before analysis. Make water-soluble As determinations on samples as received, without further pulverization or drying. In the case of lye, NaCN, or KCN, weigh large quantities in weighing bottles and analyze aliquots of the aqueous solns.

#### 6.2

##### MOISTURE—OFFICIAL

(Applicable to Paris green, London purple, powdered lead arsenate, calcium arsenate, magnesium arsenate, zinc arsenite, and powdered Bordeaux mixture.)

Dry 2 g to constant weight at 105–110° and report loss in weight as moisture.

##### TOTAL ARSENIC

##### *I. By Cuprous Chloride Distillation (1)—Official*

(Applicable except in presence of nitrates to determination of total arsenic in Paris green, lead arsenate, calcium arsenate, zinc arsenite, magnesium arsenate, and Bordeaux mixture with arsenicals.)

#### 6.3

##### REAGENTS

(a) *Standard arsenious oxide soln.*—Dissolve 2 g of pure  $\text{As}_2\text{O}_3$  in beaker by boiling with 150–200 ml of  $\text{H}_2\text{O}$  containing 10 ml of  $\text{H}_2\text{SO}_4$ , cool, transfer to 500 ml volumetric flask, and dilute to mark.

(b) *Standard iodine soln.*—Approximately 0.05 *N*. Mix 6.35 g of pure I with twice this weight of pure KI, dissolve in small quantity of  $\text{H}_2\text{O}$ , filter, and dilute filtrate to 1 liter in volumetric flask. Standardize against (a) as follows: Pipet 50 ml of the  $\text{As}_2\text{O}_3$  soln into Erlenmeyer flask, dilute to same volume as that of aliquot used for titration in actual determination, neutralize with  $\text{NaHCO}_3$ , add 4–5 g in excess, and add the standard I soln from a buret, shaking flask continuously until yellow color disappears slowly from soln. Add 5 ml of the starch indicator (e) and continue adding the I soln, dropwise, until a permanent blue color is obtained. Calculate value of the standard I soln in terms of  $\text{As}_2\text{O}_3$  and  $\text{As}_2\text{O}_5$ . For conversion of  $\text{As}_2\text{O}_3$  to  $\text{As}_2\text{O}_5$  multiply by 1.1618. Occasionally restandardize the I soln against the standard  $\text{As}_2\text{O}_3$  soln.

(c) *Standard bromate soln.*—Dissolve 1.526 g of  $\text{NaBrO}_3$  in  $\text{H}_2\text{O}$  and dilute to 1 liter; 1 ml of this soln is ca equal to 0.003 g of  $\text{As}_2\text{O}_3$ . Standardize against (a) as follows: Pipet 25 ml aliquots of the  $\text{As}_2\text{O}_3$  soln into 500 ml Erlenmeyer flasks, add 15 ml of HCl, dilute to 100 ml, heat to 90°, and titrate with the bromate soln, using 10 drops of the methyl orange indicator (f). Do not add indicator until near end of titration, and agitate liquid continuously in order to avoid local excess of the bromate soln. Add bromate soln very slowly when approaching end of titration; end point is shown by change from red to colorless.

(d) *Sodium hydroxide soln.*—Dissolve 400 g of NaOH in  $\text{H}_2\text{O}$  and dilute to 1 liter.

(e) *Starch indicator.*—Mix ca 2 g of finely powdered potato starch with cold  $\text{H}_2\text{O}$  to thin paste; add ca 200 ml of boiling  $\text{H}_2\text{O}$ , stirring constantly, and immediately discontinue heating. Add ca 1 ml of Hg, shake, and allow the starch to stand over the Hg.

(f) *Methyl orange indicator.*—Dissolve 0.5 g of methyl orange in  $\text{H}_2\text{O}$  and dilute to 1 liter.

## 6.4

## APPARATUS

Fig. 7.—Distillation flask is of 500 ml capacity and rests on metal gauze that fits over circular hole in heavy sheet of asbestos board, which in turn extends out far enough to protect sides of flask from direct flame of burner. First receiving flask holds 500 ml and contains 40 ml of  $H_2O$ ; second holds 500 ml and contains 100 ml of  $H_2O$ . Volume in first flask should not exceed 40 ml, otherwise there may be

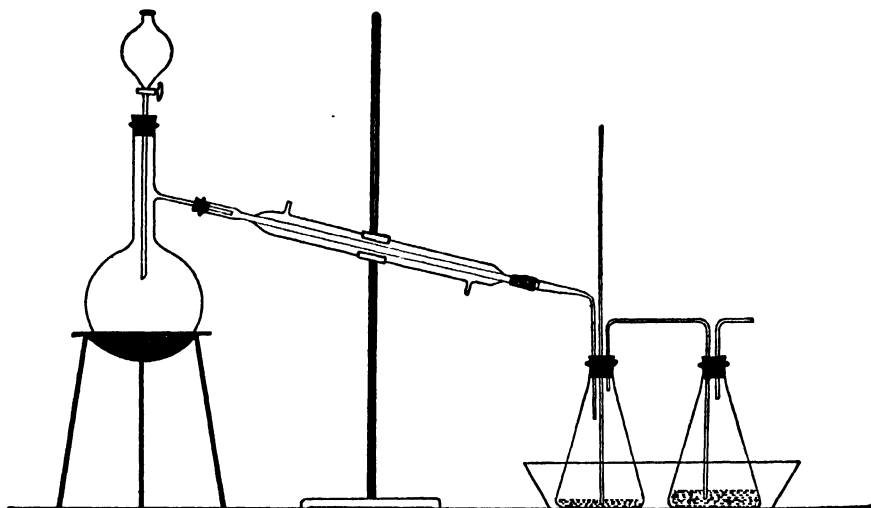


FIG. 7.—APPARATUS FOR DISTILLATION OF ARSENIOS CHLORIDE

separated a compound of As that cannot readily be redissolved without danger of loss of  $AsCl_3$ . Keep both flasks cool by placing them in pan thru which  $H_2O$  circulates, or which contains  $H_2O$  and pieces of ice.

## 6.5

## DETERMINATION

Weigh quantity of sample containing not more than 0.4 g of As and wash into distillation flask by means of 100 ml of HCl. Add 5 g of  $Cu_2Cl_2$  and distil. When volume in distillation flask is reduced to ca 40 ml add 50 ml more of HCl by means of dropping funnel and continue distillation, repeating addition of 50 ml portions of HCl until 200 ml of the acid distillate has passed over. Wash down condenser and all connecting tubes carefully, transfer these washings and contents of Erlenmeyer flasks to liter volumetric flask, dilute to mark, and mix thoroly. Titrate distillate by one of following procedures:

(a) Pipet 200 ml aliquot into Erlenmeyer flask and nearly neutralize with the NaOH soln, using few drops of phenolphthalein indicator, 2.10(d), and keeping soln well cooled. If neutral point is passed, add HCl until again slightly acid. Neutralize with  $NaHCO_3$ , add 4–5 g in excess, and add the standard I soln from buret, shaking flask continuously until yellow color disappears slowly from soln. Add 5 ml of the starch indicator and continue adding the I soln dropwise until permanent blue color is obtained.

(b) (2) Pipet 200 ml aliquot into Erlenmeyer flask and titrate with the bromate soln, 6.3(c), beginning "heat to  $90^\circ$ ."

From number of ml of standard soln used, calculate percentage of As in sample.

Report as  $\text{As}_2\text{O}_3$  or  $\text{As}_2\text{O}_5$ , according to whether the As is present in trivalent or pentavalent form. If condition of the arsenic is unknown, report as As.

## II. By Hydrazine Sulfate Distillation (3)—Official

(Nitrates do not interfere in this method. Applicable to determination of total arsenic in Paris green, lead arsenate, calcium arsenate, zinc arsenite, magnesium arsenate, and Bordeaux mixture with arsenicals.)

### 6.6

#### REAGENTS

*Hydrazine sulfate-sodium bromide soln.*—Dissolve 20 g of  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{SO}_4$  and 20 g of NaBr in 1 liter of HCl (1 + 4). See 6.3 for other reagents and solns.

### 6.7

#### APPARATUS.—See 6.4

### 6.8

#### DETERMINATION

Weigh quantity of sample containing not more than 0.4 g of As and transfer to distilling flask. Add 50 ml of the  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{SO}_4$ -NaBr soln, close flask with the stopper that carries funnel tube, and connect side tube with condenser. Boil 2–3 min., add 100 ml of HCl by means of the dropping funnel, and distil until volume in distilling flask is reduced to ca 40 ml; add 50 ml more of HCl and continue distillation until contents of flask are again reduced to ca 40 ml. Wash down condenser, transfer contents of receiving flask to a liter volumetric flask, dilute to volume, and mix thoroly. Titrate distillate—

(a) As directed under 6.5(a); or

(b) Pipet a 200 ml aliquot into an Erlenmeyer flask, add 10 ml of HCl, and titrate with the standard bromate soln, 6.3(c), beginning "heat to 90°."

From number of ml of standard soln used, calculate percentage of As in sample. Report as  $\text{As}_2\text{O}_3$  or  $\text{As}_2\text{O}_5$ , according to whether the As is present in trivalent or pentavalent form. If condition of the arsenic is unknown, report as As.

## III. (4)—Tentative

(Applicable in presence of sulfides, sulfites, thiosulfates, or large quantities of sulfur.)

### 6.9

#### REAGENT

*Sodium thiosulfate soln.*—Approximately 0.05 N. Dissolve 13 g of crystallized  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and dilute to 1 liter. See 6.3 for other reagents and solns and 6.4 for the apparatus.

### 6.10

#### DETERMINATION

Weigh 2 g of sample and transfer to distilling flask. Add soln of 5–8 g of  $\text{Cu}_2\text{Cl}_2$  in 100 ml of HCl and shake to bring sample completely in contact with the acid soln and to expel  $\text{H}_2\text{S}$ . When reaction has ceased, close flask, connect with condenser, and distil as directed under 6.5 until 200 ml of acid distillate has passed over. Make distillate to volume in liter flask, mix thoroly, and transfer 200 ml aliquot to 400 ml Pyrex beaker or porcelain casserole. Add 10 ml of  $\text{HNO}_3$  and 5 ml of  $\text{H}_2\text{SO}_4$ , evaporate to sirupy consistency on steam bath, then heat on hot plate until white fumes of  $\text{H}_2\text{SO}_4$  appear. Cool and wash into 500 ml Erlenmeyer flask. If quantity of  $\text{H}_2\text{SO}_4$  is appreciably lessened by fuming, add sufficient to make total quantity of  $\text{H}_2\text{SO}_4$  ca 5 ml. Dilute to 100–150 ml, add 1.5 g of KI, and boil until volume is reduced to ca 40 ml. Cool soln under running  $\text{H}_2\text{O}$ , dilute to 100–150 ml, and add the  $\text{Na}_2\text{S}_2\text{O}_3$  soln, 6.9, dropwise until the I color is just removed. Nearly

neutralize the  $\text{H}_2\text{SO}_4$  with the  $\text{NaOH}$  soln, 6.3(d), finish neutralization with  $\text{NaHCO}_3$ , add 4–5 g in excess, and titrate with the standard I soln as directed under 6.3(b). From number of ml of standard soln used calculate percentage of As in sample. Report as  $\text{As}_2\text{O}_3$  or  $\text{As}_2\text{O}_5$  according to whether As is present in trivalent or pentavalent form. If condition of the arsenic is unknown, report as As.

#### WATER-SOLUBLE ARSENIC—OFFICIAL

(Applicable to determination of water-soluble arsenic in lead arsenate, calcium arsenate, zinc arsenite, magnesium arsenate, and Bordeaux mixture with arsenicals.)

6.11

REAGENTS.—See 6.3 and 6.9

6.12

#### DETERMINATION

To 2 g of original sample if a powder, or 4 g if a paste, in liter Florence flask, add 1 liter of recently boiled  $\text{H}_2\text{O}$  that has been cooled to  $32^\circ$ . Stopper flask and place in water bath kept at  $32^\circ$  by means of thermostat. Digest 24 hours, shaking hourly for 8 hours during this period. Filter thru dry filter. (If filtrate is not clear, refilter thru Büchner funnel containing paper and sufficient coating of filter-cel to give a clear soln. Discard first 50 ml.) Transfer 250–500 ml of clear filtrate to Erlenmeyer flask, add 3 ml of  $\text{H}_2\text{SO}_4$ , and evaporate on hot plate. When volume reaches ca 100 ml add 1 g of KI, and continue boiling until volume is ca 40 ml. Cool, dilute to ca 200 ml, and add the  $\text{Na}_2\text{S}_2\text{O}_3$  soln, 6.9, dropwise, until I color is exactly removed. (Avoid use of starch indicator at this point.) Neutralize with  $\text{NaHCO}_3$ , add 4–5 g in excess, titrate with the standard I soln until yellow color disappears slowly, add 5 ml of the starch indicator, and continue titration to permanent blue color. Make correction for quantity of standard I soln necessary to produce the same color, using same reagents and volume. From number of ml of standard I soln used calculate percentage of water-soluble As in sample.

6.13

#### LEAD OXIDE (5)—OFFICIAL

(Applicable to such preparations as Bordeaux-lead arsenate, Bordeaux-zinc arsenite, Bordeaux-Paris green, and Bordeaux-calcium arsenate)

Weigh 1 g of powdered sample and transfer to a beaker. Add 5 ml of  $\text{HBr}$  (ca 1.38 sp. gr.) and 15 ml of  $\text{HCl}$ , and evaporate to dryness to remove As. Repeat treatment, add 20 ml more of the  $\text{HCl}$ , and again evaporate to dryness. Add to residue 25 ml of 2  $N$   $\text{HCl}$ , heat to boiling, filter immediately to remove  $\text{SiO}_2$ , and wash with boiling  $\text{H}_2\text{O}$  to volume of 125 ml. See that all  $\text{PbCl}_2$  is in soln before filtering; if it will not dissolve completely in 25 ml of 2  $N$  acid, add 25 ml additional, and dilute filtrate to 250 ml volume. Pass in  $\text{H}_2\text{S}$  until precipitation is complete. Filter, and wash precipitate thoroly with 0.5  $N$   $\text{HCl}$  saturated with  $\text{H}_2\text{S}$ . Save filtrate and washings for determination of Zn. Transfer filter paper containing the sulfides of Pb and Cu to 400 ml Pyrex beaker and completely oxidize all organic matter by heating on steam bath with 4 ml of  $\text{H}_2\text{SO}_4$  and ca 20 ml of fuming  $\text{HNO}_3$  in covered beaker. Evaporate on steam bath and then completely remove  $\text{HNO}_3$  by heating on hot plate until copious evolution of white fumes of  $\text{H}_2\text{SO}_4$  occurs. Cool, add 2–3 ml of  $\text{H}_2\text{O}$ , and again heat to fuming. Cool, add 50 ml of  $\text{H}_2\text{O}$  and 100 ml of alcohol, and let stand several hours (preferably overnight). Filter thru Gooch crucible, previously washed with  $\text{H}_2\text{O}$ , with acidified alcohol (100 parts of  $\text{H}_2\text{O}$ , 200 parts of alcohol, and 3 parts of  $\text{H}_2\text{SO}_4$ ) and with alcohol, and then dried at  $200^\circ$ . Wash the precipitate of  $\text{PbSO}_4$  in crucible ca 10 times with the acidified alcohol, and then with alcohol, to remove  $\text{H}_2\text{SO}_4$ . Dry at  $200^\circ$  to constant

weight, keeping crucible covered to prevent loss from spattering. From weight of  $\text{PbSO}_4$ , calculate percentage of  $\text{PbO}$  in sample, using factor 0.7360.

#### COPPER (6)

(Applicable to such preparations as Bordeaux-lead arsenate, Bordeaux-zinc arsenite, Bordeaux-Paris green, and Bordeaux-calcium arsenate.)

##### 6.14

##### *Electrolytic Method—Official*

Evaporate filtrate and washings from the  $\text{PbSO}_4$  precipitation, 6.13, to fuming; add a few ml of fuming  $\text{HNO}_3$  to destroy organic matter; and continue evaporation until ca 3 ml remains. Take up with ca 100 ml of  $\text{H}_2\text{O}$ , add 1 ml of  $\text{HNO}_3$ , and filter, if necessary. Wash into weighed 150 ml Pt dish and electrolyze, using rotating anode and current of ca 3 amperes. (In lieu of Pt dish a 150 ml beaker and weighed gauze cathode may be used.) After all Cu has been deposited (ca 30 min.) and while current is still flowing, wash deposit with  $\text{H}_2\text{O}$  by siphoning. Interrupt current, rinse cathode with alcohol, dry few moments in oven, and weigh. Calculate percentage of Cu in sample.

##### 6.15

##### *Thiosulfate Volumetric Method—Official*

Proceed as directed under 6.14 to point at which filtrate and washings from the  $\text{PbSO}_4$  precipitation have been treated with fuming  $\text{HNO}_3$  and evaporated to volume of ca 3 ml. Take up in ca 50 ml of  $\text{H}_2\text{O}$ , add  $\text{NH}_4\text{OH}$  in excess, and boil until excess  $\text{NH}_3$  is expelled, as shown by change of color in liquid and partial precipitation. Add 3–4 ml of 80% acetic acid, boil 1–2 min., cool, add 10 ml of a 30% KI soln, and titrate with standard thiosulfate soln, 34.41, until the brown color becomes faint. Add starch indicator, 6.3(e), and continue titration cautiously until blue color due to free I has entirely vanished. From number of ml of standard thiosulfate soln used calculate percentage of Cu in sample.

#### ZINC OXIDE (6)—OFFICIAL

(Applicable to such preparations as Bordeaux-lead arsenate, Bordeaux-zinc arsenite, Bordeaux-Paris green, and Bordeaux-calcium arsenate.)

##### 6.16

##### REAGENT

*Mercury-thiocyanate soln.*—Dissolve 27 g of  $\text{HgCl}_2$  and 30 g of  $\text{NH}_4\text{SCN}$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

##### 6.17

##### DETERMINATION

Concentrate filtrate and washings from sulfide precipitation, 6.13, by gentle boiling to ca 50 ml, and continue evaporation on steam bath to dryness. Dissolve residue in 100 ml of  $\text{H}_2\text{O}$  containing 5 ml of  $\text{HCl}$ , and add 35–40 ml of the  $\text{Hg}$ -thiocyanate reagent with vigorous stirring. Allow to stand at least an hour with occasional stirring. Filter thru a weighed Gooch crucible, wash with  $\text{H}_2\text{O}$  containing 20 ml of the  $\text{Hg}$ -thiocyanate reagent per liter, and dry to constant weight at  $105^\circ$ . From this weight calculate percentage of  $\text{ZnO}$ , using factor 0.1633.

Some Fe is usually present and during Zn determination should be in ferrous condition. In making the sulfide precipitation the  $\text{H}_2\text{S}$  should be passed into the soln for sufficient time to reduce the Fe, as well as to precipitate the Cu and Pb. The  $\text{ZnHg}(\text{SCN})_4$  precipitate normally is white, and the occluded  $\text{Fe}(\text{SCN})_3$  should not give it more than faint pink color.

## TOTAL FLUORINE

## I. Lead Chlorofluoride Method (7)—Official, First Action

## 6.18

## REAGENTS

(a) *Fusion mixture*.—Mix anhydrous  $\text{Na}_2\text{CO}_3$  and  $\text{K}_2\text{CO}_3$  in equimolecular proportions.

(b) *Lead chlorofluoride wash soln.*—Dissolve 10 g of  $\text{Pb}(\text{NO}_3)_2$  in 200 ml of  $\text{H}_2\text{O}$ ; dissolve 1 g of NaF in 100 ml of  $\text{H}_2\text{O}$  and add 2 ml of HCl; and mix these 2 solns. Allow precipitate to settle and decant supernatant liquid. Wash 4 or 5 times with 200 ml of  $\text{H}_2\text{O}$  by decantation, and then add ca 1 liter of cold  $\text{H}_2\text{O}$  to precipitate and allow to stand 1 hour or longer, with occasional stirring. Pour thru filter and use clear filtrate. (By adding more  $\text{H}_2\text{O}$  to the precipitate of  $\text{PbClF}$  and stirring, more wash soln may be prepared as needed.)

(c) *Standard silver nitrate soln.*—0.1 or 0.2 N. Standardize by titration against pure NaCl, using  $\text{K}_2\text{CrO}_4$  indicator.

(d) *Standard potassium or ammonium thiocyanate soln.*—0.1 N. Standardize by comparing with the standard  $\text{AgNO}_3$  soln under the same conditions as obtain in the determination.

(e) *Ferric indicator.*—Add to cold saturated  $\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$  soln (free from Cl) sufficient colorless  $\text{HNO}_3$  to bleach the brown color.

(f) *Bromophenol blue indicator.*—Grind 0.1 g of the powder with 1.5 ml of 0.1 N NaOH soln and dilute to 25 ml.

## 6.19

## DETERMINATION

(1) *Samples difficult to decompose such as cryolite, and others that contain aluminum or appreciable quantities of siliceous material.*—Mix 0.5 g of sample (or less if necessary to make content of F 0.01–0.10 g) with 5 g of fusion mixture and 0.2–0.3 g of powdered  $\text{SiO}_2$ , cover with 1 g of fusion mixture, and heat to fusion over Bunsen burner. (Use of blast lamp is not required as it is only necessary that the mass be fluid, and it is preferable not to heat much beyond the temp. at which it melts. If much Al is present, a uniform, clear, liquid melt cannot be obtained. There will be particles of a white solid separated in the liquid. The melt after cooling should be colorless, or at least should not have more than a gray color.)

Leach cooled melt with hot  $\text{H}_2\text{O}$  and filter into a 400 ml beaker when disintegration is complete. Return insoluble residue to Pt dish by use of jet of  $\text{H}_2\text{O}$ ; add 1 g of  $\text{Na}_2\text{CO}_3$ ; make to volume of 30–50 ml; boil for a few minutes, disintegrating any lumps with glass rod flattened on end; filter thru same paper; wash thoroly with hot  $\text{H}_2\text{O}$ ; and adjust volume of filtrate and washings to ca 200 ml. Add 1 g of ZnO dissolved in 20 ml of  $\text{HNO}_3$  (1+9), boil 2 min. with constant stirring, filter, and wash thoroly with hot  $\text{H}_2\text{O}$ . During this washing return the gelatinous mass to beaker three times and thoroly disintegrate in the wash soln because it is difficult to wash this precipitate on filter. (The mass can easily be returned to beaker by rotating funnel above beaker while cutting precipitate loose from paper with jet of wash soln.)

Add 2 drops of bromophenol blue to filtrate, and with a cover-glass almost over the beaker add  $\text{HNO}_3$  (1+4) until color just changes to yellow. Make soln slightly alkaline with 10% NaOH, and with cover-glasses on the beakers boil gently to expel  $\text{CO}_2$ . Remove from burner, add the  $\text{HNO}_3$  until color just changes to yellow and then the dilute NaOH until color just changes to blue, and add 3 ml of 10% NaCl. (Volume of soln at this point should be 250 ml.)

Add 2 ml of HCl (1+1) and 5 g of  $\text{Pb}(\text{NO}_3)_2$  and heat on steam bath. As soon

as the  $\text{Pb}(\text{NO}_3)_2$  is in soln, add 5 g of Na acetate, stir vigorously, and digest on steam bath 30 min. with occasional stirring. Allow to stand overnight, filter, and wash precipitate, beaker, and paper once with cold  $\text{H}_2\text{O}$ , then 4 or 5 times with the  $\text{PbClF}$  soln, 6.18(b), and then once more with cold  $\text{H}_2\text{O}$ .

Transfer precipitate and paper to beaker in which precipitation was made, stir paper to pulp, add 100 ml of  $\text{HNO}_3$  (5+95), and heat on steam bath until precipitate is dissolved. (5 min. is ample to dissolve this precipitate. If sample contains an appreciable quantity of sulfates the precipitate will contain  $\text{PbSO}_4$ , which will not dissolve. In such a case heat 5–10 min. with stirring and consider the  $\text{PbClF}$  to be dissolved.) Add a slight excess of 0.1 N or 0.2 N  $\text{AgNO}_3$  soln, digest on steam bath 30 min., cool to room temp. while protected from light, filter, wash with cold  $\text{H}_2\text{O}$ , and determine  $\text{AgNO}_3$  in the filtrate by titration with the standard thiocyanate soln, using 10 ml of the ferric indicator. Subtract quantity of  $\text{AgNO}_3$  found in the filtrate from that originally added. The difference will be that required to combine with the Cl in the  $\text{PbClF}$ ; from this difference calculate percentage of F in sample on basis that 1 ml of 0.1 N  $\text{AgNO}_3$  = 0.0019 g of F.

(2) *Water-soluble fluorides in presence of organic matter.*—In presence of up to 50% organic matter such as flour, pyrethrum, tobacco powder, and derris or cubé powders, which are readily decomposed without addition of powdered  $\text{SiO}_2$  and are free from or contain only small quantities of sulfates, Al, or siliceous compounds, mix 0.5 g (or less if necessary to make content of F fall between 0.01 and 0.1 g) of sample with 5 g of fusion mixture, cover with 1 g of fusion mixture, and heat to fusion over Bunsen burner. Leach cooled melt with hot  $\text{H}_2\text{O}$ , filter into a 600 ml beaker when disintegration is complete, and wash thoroly with hot  $\text{H}_2\text{O}$ . Proceed as directed under (1), 3rd par.

In presence of over 50% organic matter or organic matter that is impractical to free without preliminary ashing, such as apple peel and pulp, transfer to Pt crucible sufficient sample to be representative of the mixture and to make content of F fall between 0.01 and 0.1 g, add 15 ml of  $\text{H}_2\text{O}$  and sufficient F-free lime (0.3–0.4 g) to render mixture distinctly alkaline to phenolphthalein, mix with glass rod, and evaporate to dryness on steam bath and in oven at  $105^\circ$ . Ignite at low heat, preferably in muffle (not above  $600^\circ$ ), until organic matter is thoroly charred. Pulverize any lumps that may be present in charred ash with glass rod, mix with 5 g of the fusion mixture, and proceed as directed in (1), 1st par., beginning "cover with 1 g of fusion mixture."

(3) *Water-soluble samples in absence of organic matter and appreciable quantities of sulfates or aluminum salts.*—In the absence of organic matter or other interfering substances the fusion may be omitted and determination made on an aliquot of a water-soluble soln containing between 0.01 and 0.1 g of F, as directed in (1), 3rd par.

In the presence of Al, as in samples containing  $\text{Na}_2\text{SiF}_6$  and  $\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ , transfer sample to 400 ml beaker, dissolve in 150 ml of hot  $\text{H}_2\text{O}$ , add 6 g of the fusion mixture, and boil. Add 1 g of ZnO dissolved in 20 ml of  $\text{HNO}_3$  (1+9), boil 2 min. with constant stirring, filter into a 500 ml volumetric flask, and wash thoroly with hot  $\text{H}_2\text{O}$ . Cool to room temp. and make to volume. Transfer a 200 ml aliquot containing 0.01–0.10 g of F to a 600 ml beaker and proceed as directed in (1), 3rd par.

(4) *Sodium and magnesium fluosilicates, or samples containing appreciable quantities of sulfates (over 5%) in absence of aluminum and boron, with or without moderate quantities of organic matter.*—With large quantities of  $\text{Na}_2\text{SiF}_6$  and some of the more volatile fluosilicates, for example Mg, where there is a possibility of some of the F being evolved as  $\text{SiF}_4$  before the fusion is effected or in samples containing appreciable quantities of sulfates, distil the F as directed in 6.23, and determine F in dis-



tillate as follows: Add several drops of bromophenol blue, make alkaline with NaOH, and adjust volume to ca 250 ml by gently boiling volume down from 400 to 250 ml. Proceed as directed under (1), 3rd par., beginning "Remove from burner."

NOTES: These procedures give accurate results for quantities of F between 0.01 and 0.10 g. Below 0.01 g the results have a tendency to be slightly low and above 0.10 slightly high. A convenient sample to fuse is one that contains 0.07–0.08 g of F; too large a sample may result in incomplete fusion. Large quantities of B compounds and alkali salts retard or prevent the complete precipitation of PbClF. B has a greater effect when the quantity of F is large than when it is small. In the procedures described B has little effect, and it may be disregarded in the analysis of insecticides if the quantity of F to be precipitated is not more than 0.03 g. With some preparations containing borax or boric acid, where it is difficult to obtain a representative mixture when an extremely small sample (0.1 g) is used for analysis, a larger sample may be taken and the PbClF precipitated from an aliquot of the fusion soln. The quantity of alkali carbonates specified in the fusion and in washing of the insoluble residue is not large enough to cause low results. If sample contains S, it should be removed with CS<sub>2</sub>, and F determined on air-dried residue, allowance being made in calculations for percentage of S removed.

### II. Travers Method (Modified) (8)—Official

(Applicable in absence of B, Al, and large quantities of pyrethrum powder.)

#### 6.20

##### REAGENTS

(a) *Alcoholic potassium chloride soln.*—Dissolve 60 g of KCl in 400 ml of H<sub>2</sub>O, add 400 ml of alcohol, and test with phenolphthalein; if soln is not neutral, adjust to exact neutrality by addition of NaOH or HCl.

(b) *Standard sodium hydroxide soln.*—Approximately 0.2 N. Prepare in a manner to assure absence of carbonate.

#### 6.21

##### DETERMINATION

Treat 0.5 g of sample in small beaker with 20–25 ml of H<sub>2</sub>O. Add 0.3 g of finely divided precipitated SiO<sub>2</sub> and a few drops of methyl orange indicator. Add HCl dropwise until soln assumes an apparently permanent pink color, after which add 2 ml in excess, cover beaker with watch-glass, and boil 1 min. Cool to room temp., add 4 g of solid KCl, and stir until the latter dissolves. Add 25 ml of alcohol and let stand 1 hour with frequent stirring. Filter thru Gooch crucible containing disk of filter paper covered by medium pad of asbestos. Wash precipitate with the alcoholic KCl soln until one washing does not destroy the color made by 1 drop of 0.2 N NaOH soln and phenolphthalein (3–4 washings are usually sufficient). Transfer crucible and contents to 400 ml beaker, add 100 ml of recently boiled H<sub>2</sub>O and 1–2 ml of 1% phenolphthalein soln, heat, and titrate with the standard NaOH soln. Finish titration with the fluoride soln actively boiling. Calculate percentage of F present on the basis that 1 ml of 0.2 N NaOH soln = 0.0057 g of F.

### III. Distillation Method (9)—Tentative

(Applicable to water-soluble or water-insoluble insecticides in absence of gelatinous SiO<sub>2</sub>, B, and Al.)

#### 6.22

##### REAGENTS

(a) *Sodium alizarin sulfonate indicator.*—Dissolve 0.1 g of sodium alizarin sulfonate in 200 ml of H<sub>2</sub>O.

(b) *Hydrochloric acid (1+249).*—1 part C. P. HCl, ca 35.4%, to 249 parts of H<sub>2</sub>O.

(c) *Thorium nitrate soln.*—Approximately 0.05 *N*. Standardize in terms of g of F/ml by titrating the F obtained by distillation from standard NaF, using procedure under 6.23. In standardizing for use with Procedure (b) add 5 ml of saturated  $\text{KMnO}_4$  soln in addition to other reagents in distillation flask.

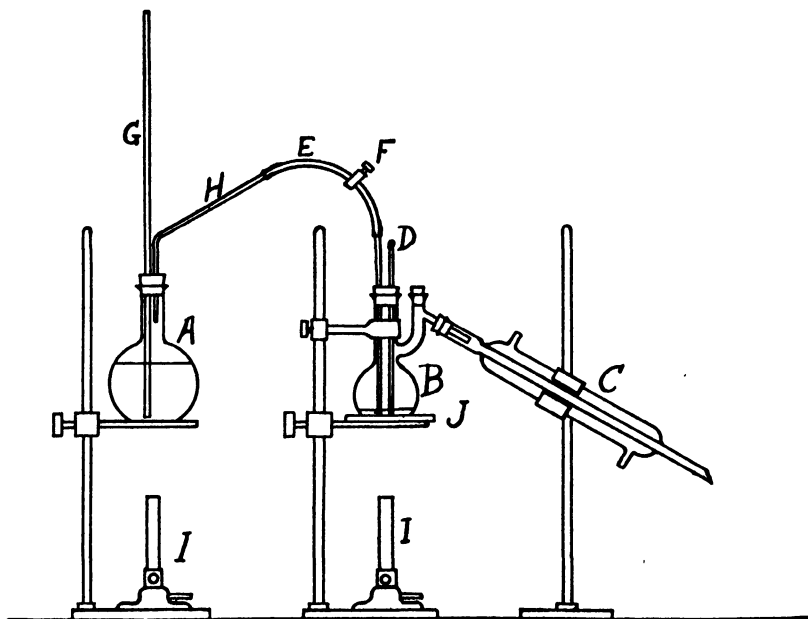


FIG. 8.—APPARATUS FOR DETERMINATION OF FLUORINE

## 6.23

### DETERMINATION

(a) *In absence of organic matter.*—Weigh quantity of sample containing ca 0.09 g of F, and with aid of a little  $\text{H}_2\text{O}$  transfer to 250 ml Claisen distillation flask containing 12 glass beads. Adjust volume to ca 30 ml and close flask with a two-holed rubber stopper, thru which passes a thermometer and a 4 mm glass tube, both of which extend down into soln. (The 4 mm glass tube extends ca 5 cm above rubber stopper and by means of rubber tube E connects still with 1 liter Florence flask containing  $\text{H}_2\text{O}$ , which is heated to generate steam. The flask is equipped with a steam discharge, H, and a pressure tube, G.

Bring  $\text{H}_2\text{O}$  in steam generating flask to b.p. with the pinchcock, F, in the release tube open. Connect distilling flask to condenser, and add 25 ml of  $\text{H}_2\text{SO}_4$  thru top of the 4 mm tube by means of pipet or special funnel. With the stopcock, F, open, connect the rubber tubing to the 4 mm tube. Light burner under Claisen flask. Regulate flow of steam by adjusting burner flames and stopcock, F, so that volume of soln is held constant and a temp. of  $145\text{--}150^\circ$  is maintained in flask B. Continue distillation until 400 ml of distillate is collected. Make to volume in 500 ml graduated flask, take one aliquot of 50 ml in a tall-form 150 ml beaker, and add 5 drops of indicator. Adjust acidity with 1% NaOH and the dilute HCl until pink color is just discharged. Add 2 ml of the HCl, and using a buret graduated in 0.05 ml titrate with 0.05 *N*  $\text{Th}(\text{NO}_3)_4$  to a permanent pink.

(b) *In presence of organic matter.*—In presence of moderate quantities of organic matter transfer quantity of sample sufficient to make F content ca 0.09 g and containing not more than 0.2 g of organic matter, with the aid of a little  $H_2O$ , to a 250 ml Claisen distillation flask containing 12 beads. Add 5 ml of saturated  $KMnO_4$  soln, adjust volume to ca 30 ml, and proceed as directed in (a), beginning "close flask with a two-holed rubberstopper." In presence of large quantities of organic matter, transfer sample to medium sized Pt dish, add 15 ml of  $H_2O$  and sufficient F-free lime to render mixture distinctly alkaline to phenolphthalein, mix with glass rod, evaporate to dryness on steam bath and in oven at  $105^\circ$ . Ignite at low heat, preferably in muffle (not above  $600^\circ$ ) until organic matter is thoroly charred. Pulverize any lumps that may be present in the charred ash with a glass rod and transfer to 250 ml Claisen distillation flask by brushing and finally wash out dish with 30 ml of 10%  $H_2SO_4$ . Except to add 22 ml instead of 25 ml of the  $H_2SO_4$ , proceed as directed in (a), par. 2, beginning "Bring  $H_2O$  in steam generating flask. . ."

NOTE: Should a coating of precipitated  $SiO_2$  form on the inside of the distillation flask, remove by treatment with hot concentrated alkali soln, as it is capable of retaining F during the distillation of some samples and giving it up, at least in part, in subsequent distillations.

#### FLUORINE PRESENT AS SODIUM FLUOSILICATE—TENTATIVE

(Boron, lime, and alum absent)

#### 6.24

##### REAGENTS

(a) *Alcoholic potassium chloride soln.*—Dissolve 60 g of KCl in 400 ml of recently boiled and cooled  $H_2O$ , and add 400 ml of neutral alcohol.

(b) *Alcoholic potassium chloride and sodium carbonate soln.*—Dissolve 1.0 g of  $Na_2CO_3$  in 100 ml of reagent (a).

(c) *Standard sodium hydroxide soln.*—Approximately 0.2 N. Assure absence of carbonate.

#### 6.25

##### DETERMINATION

Weigh 1 g of sample into Pt dish, and add rapidly with continuous stirring 50 ml of the alcoholic  $KCl-Na_2CO_3$  reagent. Do not allow the soln to become acid, and if necessary use a larger quantity of the reagent to insure alkalinity. Continue stirring until all soluble portions of sample have dissolved. Filter with suction thru Gooch crucible containing disk of filter paper covered by medium pad of asbestos. Wash precipitate with the alcoholic KCl soln until wash liquid is not alkaline to phenolphthalein. Transfer crucible and contents to 400 ml beaker, add 100 ml of recently boiled  $H_2O$  and 1–2 ml of 1% phenolphthalein soln, heat, and titrate with the standard NaOH soln. Finish titration with the F soln actively boiling. Calculate percentage of  $Na_2SiF_6$  on the basis that each ml of 0.2 N NaOH = 0.009403 g of  $Na_2SiF_6$ .

#### PARIS GREEN

#### 6.26

MOISTURE—OFFICIAL.—See 6.2

#### 6.27

TOTAL ARSENIC—OFFICIAL.—See 6.5 or 6.8

##### TOTAL ARSENIOS OXIDE

(Following methods determine only the As present in trivalent form,  $As_2O_3$ . They also determine any Sb that may be present in trivalent form,  $Sb_2O_3$ . Ferrous and cuprous salts vitiate the results.)

*Method I (10)—Official***6.28****REAGENT**

*Ammonium chloride soln.*—Dissolve 250 g of  $\text{NH}_4\text{Cl}$  in  $\text{H}_2\text{O}$  and dilute to 1 liter. For other reagents and solns see 6.3.

**6.29****DETERMINATION**

Weigh 0.3 g of sample and wash into Erlenmeyer flask with 10–15 ml of  $\text{HCl}$  (1+4) or 10–15 ml of  $\text{H}_2\text{SO}_4$  (1+4), followed by ca 100 ml of  $\text{H}_2\text{O}$ , and heat on steam bath only long enough to complete soln, at temp. not exceeding  $90^\circ$ . (If  $\text{H}_2\text{SO}_4$  is used soln may be heated to boiling.) Cool, neutralize with  $\text{NaHCO}_3$ , add 4–5 g in excess, and then add sufficient  $\text{NH}_4\text{Cl}$  soln to dissolve the precipitated  $\text{Cu}$ . Dilute somewhat and titrate as directed under 6.3(b). Make correction for quantity of I soln necessary to produce blue color with starch in presence of  $\text{Cu}$  (using an equivalent weight of  $\text{CuSO}_4$ ). From corrected number of ml of standard I soln used calculate percentage of  $\text{As}_2\text{O}_3$ .

**6.30***Method II (11)—Official*

Weigh 1.5 g of sample and wash into 250 ml volumetric flask with 100 ml of  $\text{HCl}$  (1+4), heating to maximum of  $90^\circ$ , if necessary, to secure complete soln of sample. Cool, and make to volume.

(a) Transfer 50 ml aliquot to 500 ml Erlenmeyer flask, add 10 ml of  $\text{HCl}$ , heat to  $90^\circ$ , and titrate with the standard bromate soln as directed under 6.3(c), beginning “titrate with the bromate soln.” Or,

(b) Proceed as directed under (a) but make titration without heating soln.

From number of ml of bromate soln used calculate percentage of  $\text{As}_2\text{O}_3$ .

**6.31****WATER-SOLUBLE ARSENIOUS OXIDE—OFFICIAL**

To 1 g of sample in liter Florence flask add 1 liter of recently boiled  $\text{H}_2\text{O}$  that has been cooled to  $32^\circ$ . Stopper flask and place in water bath kept at  $32^\circ$  by means of thermostat. Digest 24 hours, shaking hourly for 8 hours during this period. Filter thru dry filter and transfer 250 ml of filtrate to Erlenmeyer flask; add 4–5 g of  $\text{NaHCO}_3$  and titrate with the I soln, 6.3(b), to permanent blue color, using starch indicator, 6.3(e). Correct for quantity of I soln necessary to produce same color, using same reagents and volume. Calculate quantity of  $\text{As}_2\text{O}_3$  present and express results as percentage of water-soluble  $\text{As}_2\text{O}_3$ .

**TOTAL COPPER OXIDE****6.32***Electrolytic Method—Official*

Treat 2 g of sample in beaker with 100 ml of  $\text{H}_2\text{O}$  and ca 2 g of  $\text{NaOH}$  and boil thoroly until all  $\text{Cu}$  is precipitated as  $\text{Cu}_2\text{O}$ . Filter, wash well with hot  $\text{H}_2\text{O}$ , dissolve precipitate in hot  $\text{HNO}_3$  (1+4), cool, transfer to 250 ml volumetric flask, and dilute to mark. Electrolyze aliquot of 50 or 100 ml, as directed under 6.14. Calculate to percentage of  $\text{CuO}$ .

**6.33***Volumetric Thiosulfate Method (12)—Official*

Determine  $\text{Cu}$  in aliquot of the  $\text{HNO}_3$  soln of  $\text{Cu}_2\text{O}$ , 6.32, by titrating with standard thiosulfate soln as directed under 6.15, and calculate to percentage of  $\text{CuO}$ .

## LEAD ARSENATE

## 6.34

## MOISTURE—OFFICIAL

(a) *Powder*.—Dry 2 g to constant weight at 105–110° and report loss in weight as moisture.

(b) *Paste*.—Proceed as directed under (a), using 50 g. Grind dry sample to fine powder, mix well, transfer small portion to sample bottle, and again dry for 1–2 hours at 105–110°. Use this anhydrous material for determination of total PbO and total As.

## TOTAL ARSENIC

## 6.35

*Method I—Official—See 6.5 or 6.8*

## 6.36

*Method II (13)—Official*

(Not applicable in presence of antimony.)

Dissolve 1 g of powdered sample with  $\text{HNO}_3$  (1+4) in porcelain casserole or evaporating dish, add 5 ml of  $\text{H}_2\text{SO}_4$ , and heat on hot plate to copious evolution of white fumes. Cool, add a little  $\text{H}_2\text{O}$ , and again evaporate until appearance of white fumes to assure removal of last trace of  $\text{HNO}_3$ . Wash into 200 ml volumetric flask with  $\text{H}_2\text{O}$ , cool, dilute to volume, and filter thru a dry filter. Transfer 100 ml of filtrate to Erlenmeyer flask and proceed as directed under 6.12, beginning "add 1 g of KI." From number of ml of standard I soln used calculate percentage of total As in terms of  $\text{As}_2\text{O}_3$ .

## 6.37

## TOTAL ARSENIUS OXIDE (14)—OFFICIAL

Weigh 2 g of powdered sample and transfer to 200 ml volumetric flask, add 100 ml of  $\text{H}_2\text{SO}_4$  (1+6), and boil 30 min. Cool, dilute to volume, shake thoroly, and filter thru dry filter. Nearly neutralize 100 ml of filtrate with NaOH soln, 6.3(d), using a few drops of phenolphthalein indicator, 2.10(d). If neutral point is passed, make acid again with the dilute  $\text{H}_2\text{SO}_4$ . Continue as directed under 6.3(b), beginning "neutralize with  $\text{NaHCO}_3$ ." From number of ml of standard I soln used calculate percentage of  $\text{As}_2\text{O}_3$ .

## TOTAL ARSENIC OXIDE (15)—TENTATIVE

## 6.38

## REAGENTS

(a) *Potassium iodide soln*.—Dissolve 20 g of KI in  $\text{H}_2\text{O}$  and dilute to 100 ml.

(b) *Standard thiosulfate soln*.—Prepare ca 0.05 N soln as follows: Dissolve 13 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in recently boiled and cooled  $\text{H}_2\text{O}$ , filter, and dilute to 1 liter with recently boiled and cooled  $\text{H}_2\text{O}$ . Standardize as follows:

Dissolve ca 0.7 g of  $\text{PbHAsO}_4$  in 50 ml of HCl in Erlenmeyer flask. If necessary to effect soln, heat on steam bath, keeping flask covered with watch-glass to prevent evaporation of acid. Cool to 20–25°, add 10 ml of the KI soln, (a), and 50 ml (or more if necessary to produce clear soln) of  $\text{NH}_4\text{Cl}$  soln, 6.28, and immediately titrate liberated I with the standard  $\text{Na}_2\text{S}_2\text{O}_3$  soln. When color becomes a faint yellow, dilute with ca 150 ml of  $\text{H}_2\text{O}$  and continue titration carefully, dropwise, until colorless, using starch indicator, 6.3(e), near end point. From weight of  $\text{PbHAsO}_4$  and number of ml of  $\text{Na}_2\text{S}_2\text{O}_3$  soln used calculate value of latter in terms of  $\text{As}_2\text{O}_3$  ( $\text{As}_2\text{O}_3$  in  $\text{PbHAsO}_4 = 33.10\%$ ).

Prepare pure  $\text{PbHAsO}_4$  by pouring a soln of  $\text{Pb}(\text{NO}_3)_2$  into a soln of  $\text{KH}_2\text{AsO}_4$ , which should be in excess. Collect precipitate by filtration, dissolve it in smallest

possible quantity of boiling  $\text{HNO}_3$  (1+4), and pour soln into large quantity of  $\text{H}_2\text{O}$  (50–100 ml of the  $\text{HNO}_3$  soln in 2–3 liters of  $\text{H}_2\text{O}$ ). Collect precipitate by filtration and dry at  $110^\circ$ .

**6.39****DETERMINATION**

Weigh 0.5 g of powdered sample and transfer to Erlenmeyer flask. Add 25–30 ml of  $\text{HCl}$  and evaporate to dryness on steam bath. Add 50 ml of  $\text{HCl}$  and proceed as directed under 6.38(b), beginning "If necessary to effect soln, heat on steam bath." From number of ml of standard  $\text{Na}_2\text{S}_2\text{O}_3$  soln used calculate percentage of  $\text{As}_2\text{O}_3$ .

**6.40****WATER-SOLUBLE ARSENIC—OFFICIAL**

Proceed as directed under 6.12, and calculate results as  $\text{As}_2\text{O}_3$ .

**TOTAL LEAD OXIDE****6.41***Method I (16)—Official*

Heat in 600 ml beaker on hot plate, 0.5 g of powdered sample and ca 25 ml of  $\text{HNO}_3$  (1+4). Remove any insoluble residue by filtration. Dilute to at least 400 ml, heat nearly to boiling, and add  $\text{NH}_4\text{OH}$  to slight precipitation, then  $\text{HNO}_3$  (1+9) to redissolve precipitate, adding 1–2 ml in excess. Pipet into this soln, kept almost boiling, 50 ml of hot 10%  $\text{K}_2\text{CrO}_4$  soln, stirring constantly. Decant while hot thru weighed Gooch crucible, previously heated to  $140$ – $150^\circ$ , and wash several times by decantation and then on filter with boiling  $\text{H}_2\text{O}$  until washings are colorless. Dry the  $\text{PbCrO}_4$  at  $140$ – $150^\circ$  to constant weight. From weight of  $\text{PbCrO}_4$  calculate percentage of  $\text{PbO}$ , using factor 0.6906. (The  $\text{PbCrO}_4$  precipitate may contain a small quantity of  $\text{PbHAsO}_4$ , which will cause slightly high results, but this error rarely amounts to more than 0.1–0.2%.)

**6.42***Method II (17)—Official*

(Not applicable in presence of calcium.)

Heat in porcelain evaporating dish or casserole on hot plate, 0.5 g of powdered sample and ca 25 ml of  $\text{HNO}_3$  (1+4). Remove any insoluble residue by filtration. Add 3 ml of  $\text{H}_2\text{SO}_4$  and evaporate on hot plate until appearance of white fumes. Cool, add few ml of  $\text{H}_2\text{O}$  (to decompose any nitro-sulfuric acid formed), and again heat to fuming. Proceed as directed under 6.13, beginning "Cool, add 50 ml of  $\text{H}_2\text{O}$  and 100 ml of alcohol."

**CALCIUM ARSENATE****6.43****MOISTURE—OFFICIAL.—See 6.2****6.44****TOTAL ARSENIC—OFFICIAL.—See 6.5 or 6.8****6.45****TOTAL ARSENIOS OXIDE (18)—OFFICIAL**

(a) *Not applicable in presence of nitrates.*—Weigh 1 g of sample, transfer to 500 ml Erlenmeyer flask, and dissolve in 100 ml of  $\text{HCl}$  (1+3). Heat to  $90^\circ$  and titrate with the standard bromate soln, 6.3(c), using 10 drops of the methyl orange indicator, 6.3(f). From number of ml of standard bromate soln used calculate percentage of  $\text{As}_2\text{O}_3$ .

(b) *Applicable in presence of small quantities of nitrates.*—Proceed as directed under (a) except to make titration at room temp.

## 6.46

## WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under 6.12, and calculate results as  $As_2O_3$ . (In the testing of Ca arsenate by this method a low value for water-soluble As may not be definite assurance against plant injury in the use of the product.)

## TOTAL CALCIUM OXIDE

*Method I (18)—Official*

## 6.47

## REAGENTS

(a) *Ammonium oxalate soln.*—Dissolve 40 g of  $(NH_4)_2C_2O_4 \cdot H_2O$  in 1 liter of  $H_2O$ .

(b) *Standard potassium permanganate soln.*—Dissolve 3.161 g of  $KMnO_4$  in freshly distilled  $H_2O$  and dilute to 1 liter. Filter thru asbestos in Gooch crucible and allow to stand several days in dark place. To standardize, dissolve 0.25 g of pure  $Na_2C_2O_4$  in  $H_2O$ , add 25 ml of  $H_2SO_4$  (1+4), dilute to 200 ml, heat to ca  $70^\circ$ , and titrate with the  $KMnO_4$  soln until soln assumes faint pink color. From this titration calculate concn. of the  $KMnO_4$  soln, which should be ca 0.1 N.

## 6.48

## DETERMINATION

Dissolve 2 g of sample in 80 ml of acetic acid (1+3), transfer to 200 ml volumetric flask, dilute to volume, and filter thru dry filter. Transfer 50 ml aliquot to beaker, dilute to ca 200 ml, heat to boiling, and precipitate the Ca with the  $(NH_4)_2C_2O_4$  soln. Allow beaker to stand 3 hours on steam bath, filter soln, and wash precipitate with hot  $H_2O$ . Dissolve precipitate in 200 ml of  $H_2O$  containing 25 ml of  $H_2SO_4$  (1+4), heat to ca  $70^\circ$ , and titrate with the  $KMnO_4$  soln. From number of ml of  $KMnO_4$  soln used calculate percentage of  $CaO$ .

## 6.49

*Method II (18)—Official*

(Not applicable in presence of lead.)

Weigh 2 g of sample, transfer to beaker, add 5 ml of  $HBr$  (ca 1.38 sp. gr.) and 15 ml of  $HCl$ , and evaporate to dryness under hood to remove As. Repeat treatment, add 20 ml of  $HCl$ , and again evaporate to dryness. Take up with  $H_2O$  and a little  $HCl$ , filter into 200 ml volumetric flask, wash, and dilute to volume. Transfer 50 ml aliquot to beaker, add 10 ml of  $HCl$  and few drops of  $HNO_3$ , boil, and make slightly alkaline with  $NH_4OH$ . Let stand few minutes and filter. Dissolve precipitate in  $HCl$  (1+4), reprecipitate, filter thru same paper, and wash with hot  $H_2O$ . To combined filtrates and washings add 20 ml of acetic acid (1+3) and adjust volume to ca 200 ml. Heat to boiling, precipitate with the  $(NH_4)_2C_2O_4$  soln, 6.47(a), and allow to stand 3 hours on steam bath. Filter, and wash with hot  $H_2O$ . Ignite, and weigh as  $CaO$ ; or dissolve precipitate in 200 ml of  $H_2O$  containing 25 ml of  $H_2SO_4$  (1+4), heat to ca  $70^\circ$ , and titrate with the  $KMnO_4$  soln, 6.47(b). From weight of  $CaO$  or number of ml of  $KMnO_4$  soln used calculate percentage of  $CaO$ .

## MAGNESIUM ARSENATE

## 6.50

## MOISTURE—OFFICIAL.—See 6.2

## 6.51

## TOTAL ARSENIC—OFFICIAL.—See 6.5 or 6.8

## 6.52

## TOTAL ARSENIOS OXIDE—OFFICIAL.—See 6.37

## 6.53

## WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under 6.12, and calculate as  $As_2O_3$ .

**ZINC ARSENITE****6.54** **MOISTURE—OFFICIAL.—***See 6.2***6.55** **TOTAL ARSENIC—OFFICIAL**

Proceed as directed under 6.5 or 6.8, and calculate as  $\text{As}_2\text{O}_3$ .

**TOTAL ARSENIOS OXIDE****6.56** *Method I (18)—Official*

(a) Weigh 2 g of sample and transfer to beaker. Dissolve in 80 ml of HCl (1+4), wash into 200 ml volumetric flask, and dilute to volume. Thoroughly mix soln and filter thru dry filter. Transfer 25 ml aliquot to 500 ml Erlenmeyer flask, add 20 ml of HCl, and dilute to 100 ml. Heat to 90° and titrate with the standard bromate soln, 6.3(c). Or,

(b) Proceed as directed under (a) without heating soln.

**6.57** *Method II—Official*

Proceed as directed under 6.29, using appropriate Zn salt for blank determination.

**6.58** **WATER-SOLUBLE ARSENIC—OFFICIAL**

Proceed as directed under 6.12, and calculate results as  $\text{As}_2\text{O}_3$ .

**6.59** **TOTAL ZINC OXIDE (18)—OFFICIAL**

Transfer to beaker 25 ml aliquot of soln prepared for determination of total  $\text{As}_2\text{O}_3$ , 6.56, and add 5 ml of HCl. If there is much Fe present, reduce it by adding a little  $\text{NaHSO}_3$  and heating on steam bath until odor of  $\text{SO}_2$  has practically disappeared. Cool, dilute to ca 100 ml, and proceed as directed under 6.17, beginning "add 35–40 ml of the Hg-thiocyanate reagent with vigorous stirring."

**COPPER CARBONATE****COPPER OXIDE****6.60** *Electrolytic Method—Official*

Weigh 0.5 g of sample, transfer to 150 ml Pt dish or 150 ml beaker, and dissolve in 25 ml of  $\text{HNO}_3$  (1+4). Dilute to ca 100 ml and determine Cu by electrolysis, as directed under 6.14, beginning "electrolyze, using rotating anode and current of ca 3 amperes."

**6.61** *Thiosulfate Volumetric Method—Official*

Dissolve 0.25–0.5 g of sample in 25 ml of  $\text{HNO}_3$  (1+4), dilute to ca 50 ml, and proceed as directed under 6.15, beginning "add  $\text{NH}_4\text{OH}$  in excess."

**BORDEAUX MIXTURE****6.62** **MOISTURE—OFFICIAL**

(a) *Powder*.—Dry 2 g to constant weight at 105–110°. Report loss as moisture.

(b) *Paste*.—Heat ca 100 g in oven at 90–100° until dry enough to powder readily and note loss in weight. Powder this partially dried sample and determine remaining moisture in 2 g as directed under (a). Determine  $\text{CO}_2$  as directed under 6.64, both in original paste and in this partially dried sample. Calculate total moisture by following formula:



$$M = a + \frac{(100 - a)(b + c)}{100} - d, \text{ in which } M = \% \text{ of total moisture in original paste;}$$

$a$  = % of loss in weight of original paste during first drying;  $b$  = % of loss in weight of partially dried paste during second drying;  $c$  = % of  $\text{CO}_2$  remaining in partially dried paste after first drying; and  $d$  = % of total  $\text{CO}_2$  in original paste.

#### CARBON DIOXIDE (19)—OFFICIAL

##### 6.63

##### APPARATUS

Use a 200 ml Erlenmeyer flask closed with 2-holed stopper; in one hole fit a dropping funnel, allowing stem to extend almost to bottom of flask, and thru other hole pass outlet of a condenser that is inclined upward at angle of  $30^\circ$  from horizontal. Connect upper end of condenser with a  $\text{CaCl}_2$  tube, which in turn is connected with a double U-tube filled in middle with pumice fragments, previously saturated with  $\text{CuSO}_4$  soln (20%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) and subsequently dehydrated, and with  $\text{CaCl}_2$  at either end. Connect two weighed U-tubes for absorbing the  $\text{CO}_2$ , the first filled with porous soda-lime, and the second,  $\frac{1}{2}$  with soda-lime and  $\frac{1}{2}$  with  $\text{CaCl}_2$ , placing the  $\text{CaCl}_2$  at exit end of train. Attach a Geissler bulb, partly filled with  $\text{H}_2\text{SO}_4$ , to last U-tube to show rate of gas flow, and connect an aspirator with Geissler bulb to draw air thru apparatus. Connect an absorption tower filled with soda-lime to mouth of dropping funnel to remove  $\text{CO}_2$  from the air entering apparatus.

##### 6.64

##### DETERMINATION

Weigh into the Erlenmeyer flask 2 g of powder or 10 g of paste and add ca 20 ml of  $\text{H}_2\text{O}$ . Attach flask to apparatus, omitting the 2 weighed U-tubes, and draw  $\text{CO}_2$ -free air thru apparatus until it displaces original air. Attach weighed U-tubes as directed under 6.63, close stopcock of dropping funnel, pour into it 50 ml of  $\text{HCl}$  (1+4), reconnect with soda-lime tower, and allow the acid to flow into Erlenmeyer flask, slowly if there is much  $\text{CO}_2$ , rapidly if there is little. When effervescence diminishes, place low Bunsen flame under flask and start flow of  $\text{H}_2\text{O}$  thru condenser, allowing slow current of air to flow thru apparatus at same time. Maintain a steady but quiet ebullition and a slow air current thru apparatus. Boil a few minutes after the  $\text{H}_2\text{O}$  has begun to condense, remove flame, and continue aspiration of air at rate of about 2 bubbles per second until apparatus is cool. Disconnect weighed absorption tubes, cool in balance case, and weigh. The increase in weight is  $\text{CO}_2$ .

#### COPPER

##### 6.65

##### *Electrolytic Method—Official*

Dissolve 2 g of powdered sample in 25 ml of  $\text{HNO}_3$  (1+4), dilute to 100 ml, and electrolyze, using rotating spiral anode and current of ca 3 amperes, as directed under 6.14, beginning "Wash into weighed 150 ml Pt dish."

##### 6.66

##### *Thiosulfate Volumetric Method—Official*

Dissolve 2 g of powdered sample in ca 25 ml of  $\text{HNO}_3$  (1+4), dilute to 50 ml, add  $\text{NH}_4\text{OH}$  in excess, and heat. Without removing precipitate that has formed, boil off excess of  $\text{NH}_3$ , add 3–4 ml of acetic acid, cool, add 10 ml of 30%  $\text{KI}$  soln, and titrate as directed under 6.15, beginning "titrate with standard  $\text{Na}_2\text{S}_2\text{O}_3$  soln."

**BORDEAUX MIXTURE WITH PARIS GREEN****6.67** **MOISTURE—OFFICIAL.—See 6.62****6.68** **CARBON DIOXIDE—OFFICIAL.—See 6.64****6.69** **TOTAL ARSENIC—OFFICIAL**

Proceed as directed under 6.5 or 6.8, using 2 g of sample and calculating results as  $\text{As}_2\text{O}_3$ .

**6.70** **TOTAL ARSENIOUS OXIDE—OFFICIAL**

Proceed as directed under 6.29, using 0.5–1.0 g of sample.

**6.71** **WATER-SOLUBLE ARSENIOUS OXIDE—OFFICIAL**

Proceed as directed under 6.31, using 2 g of sample and slightly acidifying aliquot used with HCl (1+4) before adding the excess of  $\text{NaHCO}_3$ .

**COPPER****6.72** *Electrolytic Method I—Official.—See 6.14***6.73** *Electrolytic Method II (Short Method)—Official*

Dissolve 2 g of powdered sample in 150 ml beaker with 5 ml of  $\text{HNO}_3$ , add 25 ml of 3%  $\text{H}_2\text{O}_2$  soln, and warm on steam bath 5–10 min. Add 25 ml more of  $\text{H}_2\text{O}_2$  soln, dilute to 100 ml, and electrolyze, using weighed gauze cathode, a rotating paddle anode, and current of 2–3 amperes. At end of ca 20 min., add 15–20 ml more of the  $\text{H}_2\text{O}_2$  soln. After all Cu is deposited (which should not require more than 45 min.) and while current is still flowing, wash deposit with  $\text{H}_2\text{O}$  by siphoning. Interrupt current, rinse with alcohol, dry few minutes in oven, weigh, and calculate percentage of Cu. (Do not pass the current for more than 5–10 min. after all Cu is deposited without adding more of the  $\text{H}_2\text{O}_2$  soln.)

**6.74** *Thiosulfate Volumetric Method—Official.—See 6.15***BORDEAUX MIXTURE WITH LEAD ARSENATE****6.75** **MOISTURE—OFFICIAL.—See 6.62****6.76** **CARBON DIOXIDE—OFFICIAL.—See 6.64****6.77** **TOTAL ARSENIC—OFFICIAL**

Proceed as directed under 6.5 or 6.8, using 2 g of sample and calculating results as  $\text{As}_2\text{O}_5$ .

**6.78** **WATER-SOLUBLE ARSENIC—OFFICIAL**

Proceed as directed under 6.12 and calculate results as  $\text{As}_2\text{O}_5$ .

**COPPER****6.79** *Electrolytic Method—Official.—See 6.14***6.80** *Thiosulfate Volumetric Method—Official.—See 6.15***6.81** **LEAD OXIDE—OFFICIAL.—See 6.13****LEAD OXIDE AND COPPER***Electrolytic Method (20)—Official*

## 6.82

## APPARATUS

*Electrodes.*—Cathode, a cylindrical Pt electrode, either gauze or plate, ca 50 mm high and 25 mm in diam. Anode, gauze or plate, ca 50 mm high and 50 mm in diam. This electrode should be sandblasted.

## 6.83

## DETERMINATION

Weigh 1 g of powdered sample and transfer to 250 ml beaker. Add 15 ml of HCl and 5 ml of HBr, and evaporate to dryness on steam bath. Repeat treatment, and finally, to remove last traces of As, add 20 ml of the HCl and again evaporate to dryness.

To residue add 25 ml of H<sub>2</sub>O and 15 ml of HNO<sub>3</sub> and heat to boiling. Cautiously boil until most of bromides and some of chlorides are expelled (characterized by changes in color, first from brown to green, and then to blue). Evaporate to dryness on steam bath. Add 10 ml of H<sub>2</sub>O and 15 ml of HNO<sub>3</sub>, and again evaporate to dryness. Take up in 50 ml of H<sub>2</sub>O and 12 ml of HNO<sub>3</sub> and heat until all salts are in solution. (It is not necessary to filter off any siliceous material that may be present.) Dilute to 200 ml and electrolyze overnight, using current of 0.15 ampere and potential of 1.5–2 volts.

Add 15–20 ml of H<sub>2</sub>O to electrolyte and continue current a few minutes. If there is no further deposition on newly exposed surfaces of electrodes, wash them several times with H<sub>2</sub>O without breaking current. Finally break current and wash once with methyl or ethyl alcohol. Dry electrodes in oven at 105–110° 1 hour. Increase in weight of cathode represents the Cu present in sample, and increase in weight of anode represents the Pb as PbO<sub>2</sub>. From increased weight of cathode, calculate percentage of Cu. Because the PbO<sub>2</sub> as weighed is not completely anhydrous, multiply by factor 0.9267 to obtain correct weight, and then calculate percentage of PbO.

## BORDEAUX MIXTURE WITH CALCIUM ARSENATE

## 6.84

MOISTURE—OFFICIAL.—See 6.62

## 6.85

CARBON DIOXIDE—OFFICIAL.—See 6.64

## 6.86

TOTAL ARSENIC—OFFICIAL

Proceed as directed under 6.5 or 6.8, using 2 g of sample, and calculating results as As<sub>2</sub>O<sub>3</sub>.

## 6.87

WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under 6.12 and calculate results as As<sub>2</sub>O<sub>3</sub>.

## COPPER

## 6.88

*Electrolytic Method I*—Official.—See 6.14

## 6.89

*Electrolytic Method II*—Official.—See 6.73

## 6.90

*Thiosulfate Volumetric Method*—Official.—See 6.15

## SODIUM AND POTASSIUM CYANIDES

CYANOGEN (21)—OFFICIAL

## 6.91

REAGENT

*Silver nitrate soln.*—0.1 N. Standardize against pure NaCl by titration, using chromate indicator.

## 6.92

## DETERMINATION

Break sample into small lumps in mortar (do not grind). Weigh quickly ca 5 g in weighing bottle and wash into 500 ml volumetric flask containing ca 200 ml of  $H_2O$ . Add a little  $PbCO_3$  to precipitate any sulfides that may be present, dilute to mark with  $H_2O$ , mix thoroly, and filter thru dry filter. Transfer 50 ml aliquot to 400 ml beaker; add 200 ml of  $H_2O$ , 5 ml of  $NaOH$  soln (100 g/liter of  $H_2O$ ), and 10 drops of saturated  $KI$  soln (or a few crystals); and titrate to faint opalescence with the  $AgNO_3$  soln. (In making this titration, it is advantageous to have the beaker over a black surface.) From number of ml of 0.1  $N$   $AgNO_3$  soln used calculate percentage of  $CN$ . The reaction is represented by the equation:  $2NaCN + AgNO_3 = NaCN \cdot AgCN + NaNO_3$ ; hence 1 ml of 0.1  $N$   $AgNO_3$  soln = 0.005204 g of  $CN$ .

## CHLORINE (22)

## Method I—Official

## 6.93

## REAGENTS

(a) *Ammonium or potassium thiocyanate soln.*—0.1  $N$ . Adjust by titrating against the 0.1  $N$   $AgNO_3$  soln, 6.91.

(b) *Ferric indicator.*—A saturated soln of  $F_2NH_4(SO_4)_2 \cdot 12H_2O$  alum from which brown color has been removed by addition of few drops of  $HNO_3$ .

## 6.94

## DETERMINATION

Transfer a 50 ml aliquot of prepared soln, 6.92, to beaker, dilute with equal volume of  $H_2O$ , add 1–2 ml of 40%  $Cl$ -free  $HCHO$  soln, stir well, and let stand 15 min. Acidify with 5 ml  $HNO_3$  (1+1), add measured volume of 0.1  $N$   $AgNO_3$  soln, 6.91, sufficient to give an excess, stir well, filter, wash, and titrate excess of  $Ag$  in combined filtrate and washings with the 0.1  $N$  thiocyanate soln, using the ferric indicator. From number of ml of 0.1  $N$   $AgNO_3$  soln, less number of ml of thiocyanate soln used, calculate percentage of  $Cl$ .

## 6.95

## Method II (22)—Official

Transfer 50 ml aliquot of prepared soln, 6.92, to distilling flask, dilute to 100–150 ml, acidify with slight excess of acetic acid, and distil, passing vapors thru a condenser, the delivery end of which dips into a soln of  $NaOH$ , to absorb the  $HCN$ . After all the  $HCN$  has been driven off (50 ml of distillate), wash liquid remaining in distilling flask into beaker, add 5 ml of  $HNO_3$  (1+1) and then a measured volume of 0.1  $N$   $AgNO_3$  soln, 6.91, sufficient to give an excess. Stir well, filter, wash, and titrate excess of  $Ag$  in combined filtrate and washings with thiocyanate soln, 6.93(a), using ferric indicator, 6.93(b). From number of ml of 0.1  $N$   $AgNO_3$  soln, less number of ml of 0.1  $N$  thiocyanate soln used, calculate percentage of  $Cl$ .

## CALCIUM CYANIDE

## CYANOGEN (23)—OFFICIAL

## 6.96

## REAGENT

*Soda-lead soln.*—Dissolve 20 g of  $Pb$  acetate in  $H_2O$ , dilute to 1 liter, and add 200 g of  $Cl$ -free  $Na_2CO_3$ .

## 6.97

## DETERMINATION

Place ca 200 ml of  $H_2O$  in 500 ml volumetric flask and carefully dry neck of flask. Weigh ca 5 g of sample in weighing bottle and transfer to flask with least possible

exposure to air. Wash mixture down into flask and mix by whirling until soln is complete and the small quantity of  $\text{CaC}_2$  has been decomposed. Add 25 ml of the soda-lead soln, or a quantity sufficient to remove sulfides; close flask with rubber stopper; and shake thoroly, preferably 30 min. Dilute to mark, mix, and filter thru dry filter. Transfer 50 ml aliquot to 400 ml beaker and proceed as directed under 6.92, beginning "add 200 ml of  $\text{H}_2\text{O}$ ." 1 ml of 0.1  $N$   $\text{AgNO}_3$  soln = 0.005204 g of CN. To obtain percentage of  $\text{Ca}(\text{CN})_2$ , multiply percentage of CN by factor 1.7702.

#### CHLORINE (23)

6.98

##### Method I—Official

Transfer 50 ml aliquot of prepared soln, 6.97, to beaker, and proceed as directed under 6.94.

6.99

##### Method II (23)—Official

Transfer 50 ml aliquot of prepared soln, 6.97, to distilling flask, and proceed as directed under 6.95.

### SOAP

#### MOISTURE (24)

6.100

##### Xylene Distillation Method—Official

Weigh ca 20 g of sample into 300–500 ml flask; add 50 ml of xylene (technical grade is satisfactory); and, to prevent foaming, add ca 10 g of lump rosin (do not use powdered). Distil into Dean and Stark type distilling tube receiver (25) and continue distillation until no more  $\text{H}_2\text{O}$  collects in receiver. Allow contents of tube to cool to room temp., read volume of  $\text{H}_2\text{O}$  under the xylene in the tube, and from this volume calculate percentage of  $\text{H}_2\text{O}$ .

#### 6.101 POTASSIUM AND SODIUM (26)—OFFICIAL

Dissolve ca 5 g of the soap in  $\text{H}_2\text{O}$ , decompose with  $\text{HCl}$  (1+4), filter off the  $\text{H}_2\text{O}$ , and wash the fat with cold  $\text{H}_2\text{O}$ . Determine both K and Na in filtrate as directed under 12.16 and 12.17.

### MINERAL OILS

#### 6.102 UNSULFONATED RESIDUE (27)—OFFICIAL

Pipet 5 ml of the oil into Babcock cream bottle ca 15 cm (6") long (either the 9 g 50% or the 18 g 30% type). To reduce viscosity of heavy oils, warm pipet after preliminary draining by drawing it several times thru flame of Bunsen burner and drain thoroly. If greater accuracy is desired, weigh measured charge and calculate its exact volume from weight and sp. gr. of the oil. Add slowly 20 ml of 38  $N$   $\text{H}_2\text{SO}_4$ , 8.18, gently shaking or rotating bottle and taking care that temp. does not rise above  $60^\circ$ . Cool in ice water if necessary. When mixture no

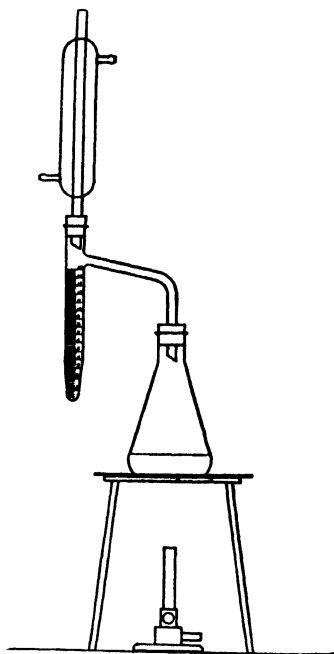


FIG. 9.—DEAN AND STARK DISTILLING TUBE RECEIVER

When mixture no

longer develops heat on shaking, agitate thoroly, place bottle in water bath, and heat at 60°–65° for 10 min., keeping contents of bottle thoroly mixed by shaking vigorously 20 seconds at 2 min. intervals. Remove bottle from bath and fill with  $\text{H}_2\text{SO}_4$  until oil rises into graduated neck. Centrifuge 5 min. (or longer if necessary to obtain a constant volume of oil) at 1200–1500 r.p.m. Read volume of unsulfonated residue from graduations on neck of bottle and, to convert to ml, multiply reading from the 9 g 50 % bottle by 0.1 and that from the 18 g 30 % bottle by 0.2. From result obtained calculate percentage by volume of unsulfonated residue.

### MINERAL OIL—SOAP EMULSIONS

#### WATER (28)

##### 6.103

##### *Xylene Distillation Method—Official*

Weigh ca 25 g of sample and proceed as directed in 6.100, except to use a smaller quantity of rosin.

##### 6.104

##### TOTAL OIL (29)—OFFICIAL

Weigh ca 10 g of sample into Babcock cream bottle, 22.68(a). Dilute with ca 10 ml of hot  $\text{H}_2\text{O}$  and add 5–10 ml of  $\text{H}_2\text{SO}_4$  (1+1). Set bottle in hot water bath ca 5 min. to hasten separation of oil, add sufficient saturated  $\text{NaCl}$  soln to bring oil layer within graduations on neck of bottle, whirl at rate of 1200 r.p.m. for 5 min., and allow to cool. Read volume of oil layer, determine its density, and from these values calculate its weight and percentage. From this percentage value, deduct percentage of fatty acids (and phenols if present) determined separately, to obtain percentage of oil.

##### 6.105

##### SOAP (28)—OFFICIAL

(Error will result if apparent molecular weight of fatty acids varies appreciably from that of oleic acid.)

Weigh 20 g of sample into a separator, add 60 ml of petroleum benzine, and extract mixture once with 20 ml and four times with 10 ml of 50% alcohol. Break emulsion if necessary with 1 or 2 ml of a 20% soln of  $\text{NaOH}$ , allowing soln to run down side of separator, which is then gently twirled and allowed to stand a few minutes. Draw off alcoholic layers and wash successively thru petroleum benzine contained in 2 other separators. Combine alcoholic extracts in beaker and evaporate on steam bath to remove alcohol. Dissolve residue in ca 100 ml of  $\text{H}_2\text{O}$  made alkaline with  $\text{NaOH}$ . Transfer to a separator, acidify with  $\text{HCl}$  or  $\text{H}_2\text{SO}_4$ , extract 3 times with ether, and wash ether extracts twice with  $\text{H}_2\text{O}$ . Combine ether extracts, evaporate in weighed beaker on steam bath, and weigh as fatty acids. From weight of fatty acids calculate percentage of soap in sample as Na- or K-oleate.

##### 6.106

##### UNSULFONATED RESIDUE—OFFICIAL

Using 5 ml of recovered oil, proceed as directed under 6.102.

##### 6.107

##### ASH (30)—OFFICIAL

Evaporate 10 g of sample, or more if necessary, in Pt dish; ignite, and leach charred mass with  $\text{H}_2\text{O}$ . Ignite residue, add leachings, evaporate to dryness, ignite, and weigh. From this weight calculate percentage of ash. Test ash for Cu, Ca,  $\text{CaF}_2$ , etc.

## TOBACCO AND TOBACCO PRODUCTS

## NICOTINE

(Including nornicotine)

*Silicotungstic Acid Method (31)—Official*

## 6.108

## REAGENT

*Silicotungstic acid soln.*—Dissolve 120 g of silicotungstic acid ( $4\text{H}_2\text{O} \cdot \text{SiO}_2 \cdot 12\text{WO}_3 \cdot 22\text{H}_2\text{O}$ ) in  $\text{H}_2\text{O}$  and dilute to 1 liter. (This acid should be white or pale yellow crystals, free from green color. The solution should be free from cloudiness and green color. Of the several silicotungstic acids,  $4\text{H}_2\text{O} \cdot \text{SiO}_2 \cdot 10\text{WO}_3 \cdot 3\text{H}_2\text{O}$  and  $4\text{H}_2\text{O} \cdot \text{SiO}_2 \cdot 12\text{WO}_3 \cdot 20\text{H}_2\text{O}$  do not give crystalline precipitates with nicotine and should not be used.)

## 6.109

## DETERMINATION

Weigh a quantity of the preparations that will contain preferably 0.1–1.0 g of nicotine. If sample contains very little nicotine (ca 0.1%) do not increase quantity to point where it interferes with distillation. Wash with  $\text{H}_2\text{O}$  into 500 ml Kjeldahl flask; and, if necessary, add a little paraffin to prevent frothing and a few small pieces of pumice to prevent bumping. Add 10 g of NaCl and 10 ml of NaOH soln (30% by weight) and close flask with rubber stopper thru which passes stem of trap bulb and inlet tube for steam. Connect by means of trap bulb to well-cooled condenser, the lower end of which dips below surface of 10 ml of HCl (1+4) in suitable receiving flask. Distil rapidly with current of steam. When distillation is well under way heat distillation flask to reduce volume of liquid as far as practicable without bumping or undue separation of insoluble matter. Distil until a few ml of distillate shows no cloud or opalescence when treated with drop of the silicotungstic acid soln and drop of HCl (1+4). Confirm alkalinity of residue in distillation flask with phenolphthalein indicator. Make distillate, which may amount to 1000–1500 ml, to convenient volume (soln may be concentrated on steam bath without loss of nicotine); mix well; and pass thru dry filter if not clear. Test distillate with methyl orange to confirm its acidity. Pipet aliquot containing ca 0.1 g of nicotine into beaker (if samples contain very small quantities of nicotine, an aliquot containing as little as 0.01 g of nicotine may be used); add to each 100 ml of liquid 3 ml of HCl (1+4), and 1 ml of silicotungstic acid for each 0.01 g of nicotine supposed to be present. Stir thoroly and let stand overnight at room temp. Before filtering, stir precipitate to see that it settles quickly and is in crystalline form. Filter either on an ashless filter or Gooch crucible and wash with HCl (1+1000) at room temp. Continue washing for 2 or 3 fillings of filter after no more opalescence appears when a few ml of fresh filtrate is tested with a few drops of nicotine distillate. In the case of a filter paper, transfer paper and precipitate to weighed Pt crucible, dry carefully, and ignite until all C is destroyed. Finally heat over Meker burner for no more than 10 min. Weight of residue  $\times 0.1141$  = weight of nicotine present in aliquot. In the case of the Gooch crucible dry in oven at  $105^\circ$  for 3 hours and weigh. Weight of residue  $\times 0.1012$  = weight of nicotine present in aliquot.

## DERRIS AND CUBÉ POWDER

## ROTENONE

## 6.110

*Crystallization Method (32)—Official*

Weigh 30 g (if sample contains more than 7% rotenone use a quantity that will give 1.0–1.5 g of rotenone in the 200 ml aliquot) of finely powdered root and 10 g

of decolorizing carbon into 500 ml glass-stoppered Erlenmeyer flask. Add 300 ml of  $\text{CHCl}_3$  measured at definite room temp.; place flask on shaking machine and fasten stopper securely. Agitate vigorously for not less than 4 hours, preferably interrupting shaking with overnight rest (or flask may be shaken continuously overnight). Filter mixture rapidly into suitable flask, using fluted paper without suction and keeping funnel covered with watch-glass to avoid loss from evaporation. Stopper flask and adjust temp. of filtrate to that of original  $\text{CHCl}_3$ .

Transfer exactly 200 ml of this soln to 500 ml Pyrex Erlenmeyer flask and distil until only ca 25 ml remains in flask. Transfer extract to 125 ml Erlenmeyer flask, using  $\text{CCl}_4$  to rinse out the 500 ml flask. Evaporate almost to dryness on steam bath in current of air. Then remove remainder of solvent under reduced pressure, heating cautiously on steam bath when necessary to hasten evaporation (suction may be applied directly to flask). Dissolve extract in 15 ml of hot  $\text{CCl}_4$  and again, in similar manner, remove all solvent. Repeat with another 10–15 ml portion of hot  $\text{CCl}_4$ . (This treatment removes all  $\text{CHCl}_3$  from the resins. The  $\text{CHCl}_3$  extract is usually completely soluble in  $\text{CCl}_4$ . If small quantities of insoluble material are present, the purification procedure described later will eliminate them. However, if large quantity of insoluble residue should remain when extract is dissolved in first portion of  $\text{CCl}_4$ , it should be filtered off and thoroly washed with further portions of hot solvent, after which the filtered soln plus washings should be treated as directed above for removal of  $\text{CHCl}_3$ .)

Add exactly 25 ml of  $\text{CCl}_4$  and heat gently to completely dissolve extract. Cool flask in ice bath several minutes and seed with a few crystals of rotenone- $\text{CCl}_4$  solvate if necessary. Stopper flask and swirl until crystallization is apparent. If at this stage only a small quantity of crystalline material separates, add an accurately weighed quantity of pure rotenone estimated to be sufficient to assure that final result, expressed as pure rotenone, is at least 1 g. Then warm to effect complete soln, and again induce crystallization. At same time prepare saturated soln of rotenone in  $\text{CCl}_4$  for washing. Place flasks containing extract and washing soln in ice bath capable of maintaining temp. of  $0^\circ$  and allow to remain overnight.

After 17–18 hours in ice bath, rapidly filter extract thru weighed Gooch crucible fitted with disk of filter paper, removing flask from ice bath only long enough to pour each fraction of extract into crucible. Rinse residue of crystalline material from flask and wash under suction with sufficient of the ice-cold saturated soln (usually 10–12 ml) to remove excess mother liquor. Allow crucible to remain under suction ca 5 min. and then dry to constant weight at  $40^\circ$  (ca an hour). The weight obtained is crude rotenone- $\text{CCl}_4$  solvate.

Break up contents of crucible with spatula, mix thoroly, and weigh 1 g into 50 ml Erlenmeyer flask. Add 10 ml of alcohol that has previously been saturated with rotenone at room temp., swirl flask a few minutes, stopper tightly, and set aside at least 4 hours, preferably overnight, at the same temp. Filter on weighed Gooch crucible fitted with disk of filter paper. Rinse crystals from flask and wash under suction with alcohol saturated with rotenone at temp. of recrystallization (ca 10 ml will usually be required). Allow crucible to remain under suction 3–5 min. and then dry at  $105^\circ$  to constant weight (ca 1 hour).

Multiply weight, expressed in grams, by weight of crude rotenone- $\text{CCl}_4$  solvate, and to product add 0.07 g, which represents correction for rotenone held in soln in the 25 ml of  $\text{CCl}_4$  used in crystallization. If any pure rotenone has been added, subtract its weight from value obtained. This gives weight of pure rotenone contained in aliquot of extract, representing 20 g of sample.

*Alternative extraction procedure.*—If sample is one in which ratio of rotenone to total extract is greater than 40%, use quantity sufficient to contain 1.0–1.5 g of



rotenone and successively extract four times with  $\text{CHCl}_3$ , using 200 ml and a period of 2 hours for second to fourth extractions. Filter after each extraction and return marc to flask for extraction with fresh solvent. Finally combine extracts, evaporate almost to dryness, and proceed as directed above, beginning at point where aliquot has been evaporated almost to dryness.

**6.111****TOTAL ETHER EXTRACT—OFFICIAL**

Extract 5 g of finely powdered root in a Soxhlet or other efficient extraction apparatus with ether for 48 hours. After extraction, concentrate extract and filter off any insoluble material that may be present. Receive filtrate in tared beaker, evaporate off ether on steam bath, and dry in oven at  $105^\circ$  to constant weight.

**PYRETHRUM POWDER****PYRETHRIN I***Mercury Reduction Method (33)—Official***6.112****REAGENTS**

(a) *Denigès reagent*.—Mix 5 g of yellow  $\text{HgO}$  with 40 ml of  $\text{H}_2\text{O}$  and, while stirring, slowly add 20 ml of  $\text{H}_2\text{SO}_4$ ; then add another 40 ml portion of  $\text{H}_2\text{O}$  and stir until completely dissolved. Test for absence of mercurous  $\text{Hg}$  by adding a few drops of (b) to 10 ml and titrating with (c) as directed under "Determination," beginning "Add 30 ml of  $\text{HCl}$ ."

(b) *Iodine monochloride soln*.—Dissolve 10 g of  $\text{KI}$  and 6.44 g of  $\text{KIO}_3$  in 75 ml of  $\text{H}_2\text{O}$  in glass-stoppered bottle; add 75 ml of  $\text{HCl}$  and 5 ml of  $\text{CHCl}_3$  and adjust to faint  $\text{I}^-$  color (in  $\text{CHCl}_3$ ) by adding dilute  $\text{KI}$  or  $\text{KIO}_3$  soln. If there is much  $\text{I}^-$  set free, use a stronger soln of  $\text{KIO}_3$  than 0.01  $M$  at first, making final adjustment with 0.01  $M$  soln. Keep in dark cupboard and readjust when necessary.

(c) *Standard potassium iodate soln*.—0.01  $M$ . Dissolve 2.14 g of pure  $\text{KIO}_3$ , previously dried at  $105^\circ$ , in  $\text{H}_2\text{O}$  and dilute to 1 liter. 1 ml of this soln = 0.0057 g of Pyrethrin I, and needs no further standardization.

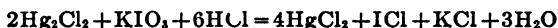
**6.113****DETERMINATION**

Extract quantity of sample that will contain 20–75 mg of Pyrethrin I (12.5–20 g) in Soxhlet or other efficient extraction apparatus 7 hours with petroleum benzine and evaporate petroleum benzine on water bath, heating no longer than necessary to remove solvent. Do not pass current of air thru flask during evaporation.

Add 15–20 ml of 0.5  $N$  alcoholic  $\text{NaOH}$  soln to flask containing pyrethrum extract, connect to reflux condenser, and boil gently 1–1.5 hours. Transfer to 600 ml beaker and add sufficient  $\text{H}_2\text{O}$  to bring volume to 200 ml. Add a few glass beads, or preferably use boiling tube and boil down to 150 ml. Transfer to 250 ml volumetric flask and add 1 g of filter-cel and 10 ml of 10%  $\text{BaCl}_2$  soln. Do not shake before making to volume. Make to volume, mix thoroly, filter off 200 ml, neutralize with  $\text{H}_2\text{SO}_4$  (1+4), and add 1 ml in excess, using 1 drop of phenolphthalein as indicator. (If necessary to have soln stand overnight at this point, it should be left in alkaline condition.) Filter thru 7 cm filter paper that has been coated lightly with suspension of filter-cel in  $\text{H}_2\text{O}$ , on Büchner funnel, and wash several times with  $\text{H}_2\text{O}$ . Transfer to 500 ml separator and extract with two 50 ml portions of petroleum benzine. Wash extracts with 2 or 3 10-ml portions of  $\text{H}_2\text{O}$ , and filter petroleum benzine extract thru plug of cotton into clean 250 ml separator. Wash cotton with 5 ml of petroleum benzine. Extract petroleum benzine with 5 ml of 0.1  $N$   $\text{NaOH}$ , shaking vigorously. Draw off aqueous layer into 100 ml beaker, wash petroleum

benzine with 5 ml of  $H_2O$  or with additional 5 ml of 0.1  $N$   $NaOH$ , and add this to the beaker. Add 10 ml of Denigès reagent to beaker and let stand 1 hour at  $25^\circ \pm 2^\circ$ . Add 20 ml of alcohol to beaker and precipitate  $HgCl$  with 3 ml of saturated  $NaCl$  soln. Warm to ca  $60^\circ$ , and filter thru small filter paper, transferring all precipitate to filter paper, and wash with 10 ml or more of hot alcohol. Wash with two or more 10 ml portions of hot  $CHCl_3$ , and place filter paper and contents in 250 ml glass-stoppered Erlenmeyer flask. Add 30 ml of  $HCl$  and 20 ml of  $H_2O$  to flask and cool; add 6 ml of  $CHCl_3$  or  $CCl_4$  and 1 ml of  $ICl$  soln and titrate with the iodate soln, shaking vigorously after each addition, until there is no iodine color in  $CHCl_3$  or  $CCl_4$  layer. From number of ml of the standard iodate soln used in titration calculate percentage of Pyrethrin I in sample.

$KIO_3$  reacts with mercurous  $Hg$  to form mercuric  $Hg$  and  $I$ . Further addition of iodate in presence of  $HCl$  oxidizes  $I$  to  $ICl$ .



Addition of  $ICl$  does not change volume relationship between mercurous  $Hg$  and iodate soln and aids in determining end point in titration of small quantities of  $Hg$ . The end point is taken when red color disappears from  $CHCl_3$  or  $CCl_4$  layer. The titration should be completed with vigorous shaking after each addition of iodate soln.

## 6.114

## PYRETHRIN II (34)—TENTATIVE

If necessary, filter aqueous residue from petroleum benzine extraction in 6.113 thru Gooch crucible. Concentrate filtrate to ca 50 ml, transfer to separator, and neutralize with  $NaHCO_3$ . Extract twice with  $CHCl_3$  and wash  $CHCl_3$  extract thru ca 15 ml of  $H_2O$  in each of two separators. Combine aqueous soln and washings; acidify strongly with  $HCl$  (ca 8 ml); saturate with  $NaCl$ , adding cautiously at first to prevent excessive ebullition of  $CO_2$ ; and extract with 50 ml of ether. Draw off aqueous layer into a second separator and extract again with 50 ml of ether. Continue this extraction and drawing off of aqueous layer, using 35 ml for third and fourth extractions. Wash the four ether extracts successively with 10 ml of  $H_2O$ , and repeat with second successive washing with another 10 ml of  $H_2O$ . Combine ether solns, draw off any  $H_2O$  that separates, and filter thru plug of cotton into 500 ml Erlenmeyer flask. Evaporate ether on water bath and dry residue at  $100^\circ$  for 10 min. Add 2 ml of neutral alcohol and 20 ml of  $H_2O$  and heat to dissolve acid. Cool, filter thru Gooch crucible if necessary, add drop or two of phenolphthalein indicator soln, and titrate with 0.02  $N$   $NaOH$  soln, of which 1 ml = 0.00374 g of Pyrethrin II.

## PYRETHRUM EXTRACTS IN MINERAL OIL

## PYRETHRIN I

*Mercury Reduction Method (33)—Tentative*

## 6.115

## REAGENTS.—See 6.112

## 6.116

## DETERMINATION

Weigh or measure a quantity of sample that will contain 20–75 mg of Pyrethrin I, and transfer to 300 ml Erlenmeyer flask.

Add 20 ml, or more if necessary, of normal alcoholic  $NaOH$  soln to flask containing pyrethrum extract, connect to reflux condenser, and boil gently 1–1.5 hours. Transfer to 600 ml beaker and add sufficient  $H_2O$  to make aqueous layer 200 ml. If more than 20 ml of alcoholic  $NaOH$  soln has been used, add sufficient  $H_2O$  so that all alco-

hol will be removed when volume has been reduced to 150 ml. Add a few glass beads, or preferably use boiling tube, and boil aqueous layer down to 150 ml. Transfer contents of beaker to 500 ml separator and draw off aqueous layer into 250 ml volumetric flask. Wash oil layer once with  $H_2O$  and add wash  $H_2O$  to aqueous portion. If slight emulsion still persists after drawing off aqueous layer and washings, it may be broken by addition of 2–3 ml of 10%  $BaCl_2$  soln. Do not shake vigorously after adding the  $BaCl_2$ , because reversed emulsion that is difficult to separate may be formed. To aqueous soln in the 250 ml flask, add 1 g of filter-cel and 10 ml or more of the  $BaCl_2$  soln. Do not shake before making to volume. Make to volume, mix thoroly, and filter off 200 ml. Test filtrate with  $BaCl_2$  to see if sufficient has been added to obtain clear soln. Neutralize with  $H_2SO_4$  (1+4) and add 1 ml in excess, using 1 drop of phenolphthalein as indicator. From this point proceed as directed under 6.113, beginning "Filter thru 7 cm filter paper."

NOTE: Chrysanthemum monocarboxylic acid reacts with Denigès reagent to form a series of colors beginning with phenolphthalein red, which gradually changes to purple, then blue, and finally to bluish green. The color reaction is very distinct with 5 mg of monocarboxylic acid and quantities as low as 1 mg can usually be detected. Therefore no Pyrethrin I should be reported if color reaction is negative.

In the analysis of samples containing much perfume or other saponifiable ingredients, it may be necessary to use as much as 50 ml of normal alcoholic NaOH. When lethanes are present, after washing the  $HgCl$  precipitate with alcohol and  $CHCl_3$ , wash once more with alcohol and then several times with hot  $H_2O$ .

## FORMALDEHYDE

### FORMALDEHYDE IN SOLUTIONS

#### *Hydrogen Peroxide Method (35)—Official*

#### 6.117

#### REAGENTS

(a) *Sulfuric acid*.—1 *N*. Prepare as directed under 2.22(b).

(b) *Sodium hydroxide soln*.—1 *N*. Standardize against (a), using litmus or bromothymol blue indicator. 1 ml = 30.3 mg of  $HCHO$ .

(c) *Hydrogen peroxide soln*.—Commercial, containing ca 3% of  $H_2O_2$ . If acid, neutralize with the NaOH (b), using litmus or bromothymol blue indicator.

(d) *Litmus indicator*.—A soln of purified litmus of such concentration that 3 drops will impart a distinct blue color to 50 ml of  $H_2O$ .

(e) *Bromothymol blue indicator*.—Dissolve 1 g of bromothymol blue in 500 ml of alcohol, 50% by volume.

#### 6.118

#### DETERMINATION

Measure 50 ml of the NaOH soln into 500 ml Erlenmeyer flask and add 50 ml of the  $H_2O_2$ . Add weighed quantity, ca 3 g, of sample, allowing point of weighing pipet to reach nearly to liquid in flask. Place funnel in neck of flask and heat on steam bath 5 min., shaking occasionally. Remove from steam bath, wash funnel with  $H_2O$ , cool flask to room temp., and titrate excess NaOH with the 1 *N* acid, using the bromothymol blue or litmus indicator. (It is necessary to cool flask before titration with the acid to obtain a sharp end point with the litmus.) From amount of 1 *N* NaOH consumed and weight of sample calculate percentage of  $HCHO$  according to following equation:



If the  $HCHO$  soln contains an appreciable quantity of free acid, titrate a separate

portion and calculate acidity as percentage of  $\text{HCOOH}$ . Make correction for this acidity in calculating percentage of  $\text{HCHO}$ .

**6.119****Cyanide Method (36)—Official**

(Applicable only to dilute solutions.)

Treat 15 ml of 0.1  $N$   $\text{AgNO}_3$  soln, 6.91, with 6 drops of  $\text{HNO}_3$  (1+1) in 50 ml volumetric flask, add 10 ml of  $\text{KCN}$  soln (3.1 g  $\text{KCN}$  dissolved in 500 ml of  $\text{H}_2\text{O}$ ), dilute to mark, shake well, filter thru dry filter, and titrate 25 ml of filtrate with 0.1  $N$   $\text{NH}_4\text{SCN}$ , 6.93(a), as directed under 12.44. Acidify another 15 ml portion of 0.1  $N$   $\text{AgNO}_3$  with 6 drops of the dilute  $\text{HNO}_3$  and treat with 10 ml of the  $\text{KCN}$  soln to which has been added a measured quantity (weight must be calculated from sp. gr.) of sample containing not over 25 mg of  $\text{HCHO}$ . Dilute to 50 ml, filter, and titrate 25 ml aliquot with the 0.1  $N$   $\text{NH}_4\text{SCN}$  for excess of  $\text{Ag}$  as before. Difference between ml of  $\text{NH}_4\text{SCN}$  used in these 2 titrations  $\times 2 =$  ml of 0.1  $N$   $\text{NH}_4\text{SCN}$  corresponding to  $\text{KCN}$  used by the  $\text{HCHO}$ . Calculate percentage of  $\text{HCHO}$  present. 1 ml of 0.1  $N$   $\text{NH}_4\text{SCN} = 3$  mg of  $\text{HCHO}$ .

**6.120****FORMALDEHYDE IN SEED DISINFECTANTS (37)—OFFICIAL**

(Applicable to determination of formaldehyde absorbed in an inert carrier, such as Bentonite, talc, charcoal, sawdust, etc.)

Weigh ca 5 g of sample containing 0.3–0.5 g of  $\text{HCHO}$  in a weighing bottle and transfer to 800 ml Kjeldahl flask. Add 25 ml of  $\text{H}_2\text{O}$  and 12 ml of  $\text{H}_2\text{SO}_4$  (1+4). Distil rapidly with steam, passing vapors thru a condenser, the delivery end of which dips into 25 ml of  $\text{H}_2\text{O}$  contained in a 500 ml volumetric flask. Continue distillation until ca 450 ml has passed over, keeping volume in distilling flask nearly constant with aid of small flame. After distillation, wash delivery tube, and dilute the distillate to volume with  $\text{H}_2\text{O}$ .

Into each of two 200 ml volumetric flasks, measure 20 ml of 0.1  $N$   $\text{AgNO}_3$ . Add to each flask 12 drops of  $\text{HNO}_3$  (1+1) and 30 ml of  $\text{H}_2\text{O}$ . To one of the flasks add slowly with constant shaking 30 ml of  $\text{KCN}$  soln (3.1 g of  $\text{KCN}$  in 1000 ml of  $\text{H}_2\text{O}$ ). Dilute to volume, shake well, and filter thru dry filter. To 100 ml of filtrate, add 3 ml of  $\text{HNO}_3$  and 5 ml of  $\text{FeNH}_4(\text{SO}_4)_2$  indicator, and titrate with 0.1  $N$   $\text{KCNS}$ .

Pipet 25 ml of the  $\text{HCHO}$  distillate into small beaker containing 30 ml of the  $\text{KCN}$  soln, mix well, and add slowly with constant shaking to the second flask containing the acidified  $\text{AgNO}_3$  soln. Dilute to volume with  $\text{H}_2\text{O}$ , filter, acidify 100 ml of filtrate with 3 ml of the  $\text{HNO}_3$ , and titrate with the  $\text{KCNS}$  soln, using the  $\text{FeNH}_4(\text{SO}_4)_2$  indicator.

The difference between the number of ml of  $\text{KCNS}$  soln used in these two titrations, multiplied by 2, gives number of ml of 0.1  $N$   $\text{KCNS}$  equivalent to the  $\text{HCHO}$ . Calculate percentage of  $\text{HCHO}$  present. 1 ml of 0.1  $N$   $\text{KCNS} = 0.003002$  g of  $\text{HCHO}$ .

**LIME-SULFUR SOLUTIONS****TOTAL SULFUR (38)—OFFICIAL**

(Sulfur-free reagents should be used)

**6.121****PREPARATION OF SAMPLE**

Weigh ca 10 g of soln, transfer to 250 ml volumetric flask, and immediately dilute to mark with recently boiled and cooled  $\text{H}_2\text{O}$ . Mix thoroly and transfer to number of small bottles, filling them completely and avoiding contact of soln with air as much as possible. Stopper bottles, seal with paraffin, and preserve in dark, cool place.

## 6.122

## DETERMINATION

Dissolve 2–3 g of  $\text{Na}_2\text{O}_2$  in 50 ml of cold  $\text{H}_2\text{O}$  in 250 ml beaker. Transfer 10 ml aliquot of the prepared soln to this aqueous soln of  $\text{Na}_2\text{O}_2$ , keeping tip of pipet constantly just under surface of liquid until necessary to raise it for drainage at end. Use clean dry pipet for measuring each portion. Cover beaker with watch-glass and heat on steam bath, with occasional stirring, until all S is oxidized to sulfate (indicated by disappearance of yellow color). Wash off watch-glass and sides of beaker, acidify with  $\text{HCl}$  (1+4), evaporate to complete dryness, treat with  $\text{H}_2\text{O}$  acidified with  $\text{HCl}$ , boil, and filter to remove  $\text{SiO}_2$ . Dilute filtrate to 300 ml, add 50 ml of  $\text{HCl}$ , heat to boiling, and add 10%  $\text{BaCl}_2$  soln (11 ml for 1 g of  $\text{BaSO}_4$ ) with constant stirring, at such rate that ca 4 min. is required for running in necessary quantity. (Rate may be regulated by attaching suitable capillary tip to buret containing the  $\text{BaCl}_2$  soln.) Evaporate to dryness on steam bath, take up with hot  $\text{H}_2\text{O}$ , filter thru quantitative filter, wash until free from chlorides, ignite carefully, and heat to constant weight over Bunsen burner. Calculate percentage of S from weight of  $\text{BaSO}_4$ , using factor 0.1374.

## MONOSULFIDE EQUIVALENT (59)—TENTATIVE

## 6.123

## REAGENT

*Iodine soln.*—0.1 *N*. Prepare as directed under 6.3(b), using 12.7 g of I and 25 g of KI per liter.

## 6.124

## DETERMINATION

Dilute 10 ml of prepared soln, 6.121, to ca 30 ml with recently boiled and cooled  $\text{H}_2\text{O}$  and titrate with the 0.1 *N* I soln until yellow color just disappears. (There should be no difficulty in determining this end point; if there is, a small crystal of Na nitroprusside may be used, but it must not be added until end point is practically reached, because the blue color, if well developed, cannot be destroyed except by excess of I.) From number of ml of 0.1 *N* I soln used calculate percentage of monosulfide equivalent. 1 ml of 0.1 *N* I = 0.001603 g of S as monosulfide equivalent.

## THIOSULFATE SULFUR

*Zinc Chloride Method (58)—Official*

## 6.125

## REAGENT

*Ammoniacal zinc chloride soln.*—Dissolve 50 g of pure  $\text{ZnCl}_2$  in ca 500 ml of  $\text{H}_2\text{O}$ , add 125 ml of  $\text{NH}_4\text{OH}$  and 50 g of  $\text{NH}_4\text{Cl}$ , and dilute to 1 liter.

## 6.126

## DETERMINATION

To 50 ml of  $\text{H}_2\text{O}$  in 200 ml volumetric flask, add, in manner indicated under 6.122, 50 ml of soln prepared as directed under 6.121. Add slight excess of the ammoniacal  $\text{ZnCl}_2$  soln and dilute to mark. Shake thoroly and filter thru dry filter. To 100 ml of filtrate add few drops of methyl orange or methyl red indicator, 6.3(f) or 2.22(i), and exactly neutralize with 0.1 *N*  $\text{HCl}$ . Titrate the neutral soln with 0.05 *N* I soln, 6.3(b), using few drops of starch indicator, 6.3(e). From number of ml of I soln used calculate percentage of thiosulfate S present. As value of the I soln is given in terms of  $\text{As}_2\text{O}_3$ , multiply this value by 1.296 to obtain equivalent of thiosulfate S.

## 6.127

*Iodine Titration Method (59)—Tentative*

Continue titration of soln used in determination of the monosulfide equivalent,

**6.124**, with the 0.1 *N* I soln, letting the I act as its own indicator until a small drop produces a slight permanent coloration. From number of ml of 0.1 *N* I used calculate percentage of thiosulfate S. 1 ml of 0.1 *N* I = 0.006412 g of S as thiosulfate.

#### SULFIDE SULFUR

**6.128**

##### *Zinc Chloride Method (38)—Official*

To 10–15 ml of H<sub>2</sub>O in small beaker, add in manner indicated under **6.122**, 10 ml aliquot of the soln prepared as directed under **6.121**. Calculate quantity of ammoniacal ZnCl<sub>2</sub> soln, **6.125**, necessary to precipitate all the S in the aliquot and add slight excess. Stir thoroly, filter, wash precipitate twice with cold H<sub>2</sub>O, and transfer filter paper and precipitate to beaker in which precipitation was made. Cover with H<sub>2</sub>O, disintegrate paper with glass rod, and add ca 3 g of Na<sub>2</sub>O<sub>2</sub>, keeping beaker well covered with watch-glass. Warm on steam bath with frequent shaking until all S is oxidized to sulfate, adding more Na<sub>2</sub>O<sub>2</sub> if necessary. Make slightly acid with HCl (1+4), filter to remove shreds of filter paper, wash thoroly with hot H<sub>2</sub>O, and determine S in filtrate as directed under **6.122**.

**6.129**

##### *Iodine Titration Method (39)—Tentative*

Allow soln from **6.127** to stand several hours with occasional stirring, or acidify with few drops of HCl (1+4); warm gently with stirring, filter, and wash thoroly with warm H<sub>2</sub>O. Place filter paper with S in a small vessel and dissolve S in ca 15 ml of NaOH soln, **6.3(d)**, by heating gently on steam or water bath 1–1.5 hours (do not boil). Keep flask covered and shake gently few times during digestion to remove S from sides. Oxidize by adding 2–3 g of Na<sub>2</sub>O<sub>2</sub> dissolved in 50 ml of cold H<sub>2</sub>O and complete determination as directed under **6.122**, beginning "Cover beaker with watch-glass."

**6.130**

##### *Indirect Method—Tentative*

The difference between total S and the sum of thiosulfate S and sulfate S is sulfide S.

#### SULFATE SULFUR

**6.131**

##### *Zinc Chloride Method—Official*

Slightly acidify soln from determination of thiosulfate S, **6.126**, with HCl (1+4), heat to boiling, add slowly and with constant stirring slight excess of 10% BaCl<sub>2</sub> soln, boil 30 min., allow to stand overnight, and filter. Calculate S from weight of BaSO<sub>4</sub>, and report as percentage of sulfate S.

**6.132**

##### *Iodine Titration Method (39)—Tentative*

To filtrate from determination of thiosulfate S, **6.127**, add several drops of HCl, precipitate in cold with 5 ml of 10% BaCl<sub>2</sub> soln, allow to stand overnight, and filter. Calculate S from weight of BaSO<sub>4</sub>, and report as percentage of sulfate S.

**6.133**

##### **TOTAL LIME (38)—OFFICIAL**

To 25 ml of prepared soln, **6.121**, add 10 ml of HCl, evaporate to dryness on steam bath, treat with H<sub>2</sub>O and few ml of HCl (1+4), warm until all CaCl<sub>2</sub> is dissolved, and filter to remove S and any SiO<sub>2</sub> that may be present. Dilute filtrate to volume of 200–250 ml, heat to boiling, and add few ml of NH<sub>4</sub>OH in excess, and then excess of saturated soln of (NH<sub>4</sub>)<sub>2</sub>C<sub>2</sub>O<sub>4</sub>. Continue boiling until precipitated CaC<sub>2</sub>O<sub>4</sub> assumes well defined granular form, allow to stand an hour, filter, and wash few times

with hot  $\text{H}_2\text{O}$ . Ignite in a Pt crucible over blast lamp to constant weight and calculate to percentage of  $\text{CaO}$ .

### ANT POISONS AND RODENTICIDES (40)

#### 6.134

#### THALLOUS SULFATE—OFFICIAL

Weigh quantity of sample containing 0.1–0.15 g of  $\text{Tl}_2\text{SO}_4$  (usually 10 g), transfer to 800 ml Kjeldahl flask, and add 25 ml of  $\text{H}_2\text{SO}_4$  followed by 5–10 ml of  $\text{HNO}_3$ . After first violent reaction has ceased, heat on Kjeldahl digestion apparatus until white fumes of  $\text{H}_2\text{SO}_4$  are evolved. Add a few drops of fuming  $\text{HNO}_3$  and continue the heating and addition of  $\text{HNO}_3$  until organic matter has been destroyed, as evidenced by colorless or light yellow soln. Cool, add 10–15 ml of  $\text{H}_2\text{O}$ , again cool, and wash contents of flask into 400 ml beaker, continuing the washing until volume is 60–70 ml. Boil several minutes to remove all  $\text{HNO}_3$ , cool, and filter into 400 ml beaker. Wash with hot  $\text{H}_2\text{O}$  until volume in beaker is 175 ml, neutralize with  $\text{NH}_4\text{OH}$ , and then slightly acidify with  $\text{H}_2\text{SO}_4$  (1+4). Add 1 g of  $\text{NaHSO}_3$  to insure reduction of the Tl from thallic to thalious form. Heat to boiling, add 50 ml of 10% KI soln, stir, and let stand overnight. Filter thru a tight Gooch crucible containing two disks of S & S 589 white ribbon filter paper covered by a medium pad of asbestos. Wash 4 or 5 times with 10 ml portions of 1% KI soln, and finally with absolute alcohol. Dry to constant weight at  $105^\circ$  (1–1½ hours in oven), and weigh as thalious iodide, TII. From this weight calculate the percentage of thallium as thalious sulfate,  $\text{Tl}_2\text{SO}_4$ , using the factor 0.7619.

### ORGANIC MERCURIAL SEED DISINFECTANTS

#### MERCURY

#### *Volatilization Method (41)—Official*

#### 6.135

#### APPARATUS

The apparatus (Fig. 10) consists of 2 flanged crucibles that can be clamped mouth to mouth by means of 2 rings and screws. The lower crucible is made of Fe and the upper one of Au. The opening of the Au crucible is slightly larger than that of the other, so that there will be no tendency for the Hg to lodge in the joint between the two flanges. The Au crucible is fitted with a cooling device by which  $\text{H}_2\text{O}$  may be slowly circulated thru large tube attached to it by Gooch tubing. The assembled apparatus rests on an asbestos board having a hole just large enough to receive crucible.

#### 6.136

#### DETERMINATION

Weigh 1 g of sample into the Fe crucible and mix thoroly with 5 g of anhydrous  $\text{Na}_2\text{CO}_3$ . Cover mixture with thin layer of  $\text{Na}_2\text{CO}_3$  and then with 10 g of finely powdered  $\text{BaCO}_3$ . Put weighed Au crucible in place, clamp the two together, set Fe crucible in place in asbestos board, start cooling  $\text{H}_2\text{O}$ , and gently heat Fe crucible. Do not run  $\text{H}_2\text{O}$  too fast because Hg amalgamates best with Au crucible if temp. is allowed to rise to ca  $50^\circ$ . Heat below red heat 30 min., cool, remove Au crucible, wash it with alcohol, dry with heat of hand, and place in  $\text{CaCl}_2$  desiccator until it attains constant weight. Calculate increase in weight of Au crucible as percentage of metallic Hg in sample. If product contains more than 12% Hg, use less than 1 g because the Au crucible can safely retain ca 0.12 g of Hg. Remove Hg from the Au crucible, preparatory to another experiment, by short ignition at dull red heat under hood having good draught. (The crucible will melt in the full heat of Bunsen burner.)

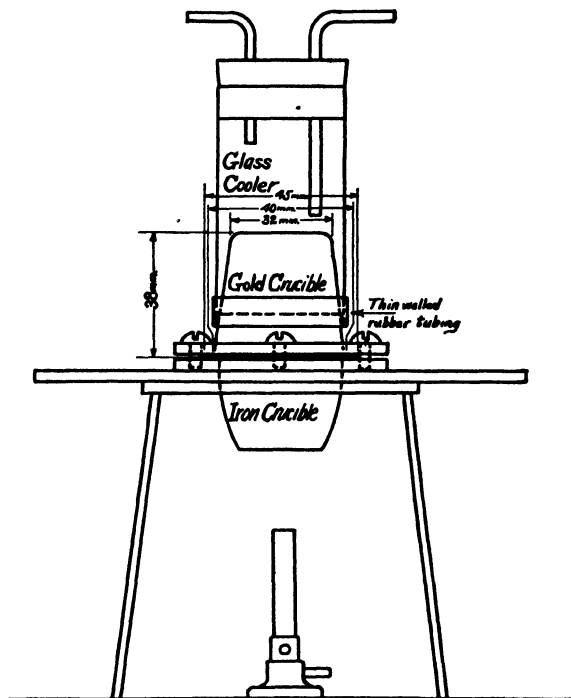


FIG. 10.—APPARATUS USED IN VOLATILIZATION METHOD FOR DETERMINATION OF MERCURY

*Precipitation Method (42)—Official*

**6.137**

**REAGENT**

*Hydrogen peroxide soln.*—30%. Commonly designated as “perhydrol” or “super-oxol.”

**6.138**

**DETERMINATION**

Place 0.5–2.0 g of sample, depending on quantity of Hg present, in 200 ml Erlenmeyer flask fitted with air condenser by means of ground-glass joint. Add 10 ml of  $\text{H}_2\text{SO}_4$ , connect flask to condenser, and rotate in order to bring all the sample into contact with the acid. Add dropwise thru condenser tube 3–5 ml of the  $\text{H}_2\text{O}_2$  soln, and mix by rotation of flask. After active reaction has subsided, heat over low flame 15–20 min., add 5 ml more of the  $\text{H}_2\text{O}_2$ , and continue heating until all organic matter is destroyed (indicated by a clear soln), adding more  $\text{H}_2\text{O}_2$  if necessary. Remove flask from heat, allow to cool, wash down condenser, and transfer contents to beaker, filtering if necessary. Dilute to ca 200 ml and destroy excess of  $\text{H}_2\text{O}_2$  by titration with  $\text{KMnO}_4$  soln. Precipitate the Hg with  $\text{H}_2\text{S}$ , filter thru weighed Gooch crucible, and dry precipitate in oven at 105–110°. Extract dried precipitate with  $\text{CS}_2$  to remove any precipitated S, again dry, and weigh. From weight of  $\text{HgS}$  calculate percentage of metallic Hg, using factor 0.8622.



## SODIUM HYPOCHLORITE SOLUTIONS (43)

## SODIUM HYPOCHLORITE

*Arsenious Oxide Titration Method—Official*

## 6.139

## REAGENTS

(a) *Arsenious oxide soln.*—0.1 *N*. Dissolve exactly 2.473 g of pure  $\text{As}_2\text{O}_3$  in a beaker by boiling with 150–200 ml of  $\text{H}_2\text{O}$  containing 10 ml of  $\text{H}_2\text{SO}_4$ . Cool, add phenolphthalein indicator, neutralize acid with  $\text{NaOH}$  soln, adjust to faint acid reaction, transfer to a 500 ml volumetric flask, and dilute to mark.

(b) *Standard iodine soln.*—Prepare as directed under 6.3(b). Standardize against (a).

## 6.140

## DETERMINATION

Transfer 20 ml aliquot of sample to liter volumetric flask and dilute to volume. Pipet 50 ml aliquot of mixture into 200 ml Erlenmeyer flask. Add the standard  $\text{As}_2\text{O}_3$  soln in excess and then add decided excess of  $\text{NaHCO}_3$ . Titrate excess of  $\text{As}_2\text{O}_3$  with the standard I soln, using starch soln, 6.3(e), or the I as indicator. Subtract volume of I soln, corrected to 0.1 *N*, from volume of  $\text{As}_2\text{O}_3$  soln used, and from this value and sp. gr. of soln, calculate percentage of  $\text{NaOCl}$ . 1 ml of 0.1 *N*  $\text{As}_2\text{O}_3$  soln = 0.003723 g of  $\text{NaOCl}$ .

## 6.141

## AVAILABLE CHLORINE—OFFICIAL

Calculate percentage of available Cl from titration described under 6.140. 1 ml of the 0.1 *N*  $\text{As}_2\text{O}_3$  = 0.003546 g of available Cl.

## 6.142

## CHLORIDE CHLORINE—OFFICIAL

Pipet 50 ml aliquot of prepared soln, 6.140, into 200 ml Erlenmeyer flask and add slight excess of the  $\text{As}_2\text{O}_3$  soln, 6.139(a), calculated from  $\text{NaOCl}$  titration; add slight excess of  $\text{HNO}_3$ , neutralize soln with  $\text{CaCO}_3$ , and titrate with 0.1 *N*  $\text{AgNO}_3$ , 6.91, using  $\text{K}_2\text{CrO}_4$ , 2.47(b), or the  $\text{Na}_3\text{As}_4\text{O}_{14}$  formed in the soln as indicator. Run blank determination on reagents and make correction for any Cl found. From this corrected titration and sp. gr. of sample calculate percentage of Cl. From this value subtract one-half the percentage of available Cl. Difference is percentage of chloride Cl.

## 6.143

## SODIUM HYDROXIDE—OFFICIAL

Pipet 25 ml of sample into a 250 ml volumetric flask, and add sufficient  $\text{H}_2\text{O}_2$  soln (neutral to phenolphthalein) to destroy  $\text{NaOCl}$ . Mix well and add sufficient neutral 10%  $\text{BaCl}_2$  soln to precipitate carbonates, make to volume, mix thoroly, and filter thru dry filter. Pipet 50 ml of filtrate into Erlenmeyer flask and titrate with 0.1 *N*  $\text{HCl}$ , using phenolphthalein as indicator, 7.6(a). From this titration and sp. gr. of sample calculate percentage of  $\text{NaOH}$ .

## CARBON DIOXIDE—OFFICIAL

## 6.144

## APPARATUS

Connect an evolution flask, to which is attached a dropping funnel protected by a tube containing soda lime, to a condenser or Kjeldahl distilling trap, which in turn is connected to 2 wash bottles containing 10%  $\text{KI}$  soln. Use glass beads or other device in wash bottles to cause gas to flow slowly thru liquid. End train with Meyer absorption tube containing 0.1 *N*  $\text{Ba}(\text{OH})_2$  soln.

## 6.145

## DETERMINATION

Pipet suitable aliquot of sample (5–20 ml, governed by quantity of  $\text{CO}_2$  present) into evolution flask, and attach flask to train. Place 50 ml of 0.1 *N*  $\text{Ba}(\text{OH})_2$  soln in the Meyer tube, and add 35–50 ml of  $\text{H}_2\text{O}_2$  soln (or sufficient quantity to reduce hypochlorite) thru dropping funnel into evolution flask. After action due to the  $\text{H}_2\text{O}_2$  has ceased, add 30 ml of  $\text{HCl}$  (1+3), heat flask to boiling, and draw air slowly thru apparatus. (Evolved gases will be freed from  $\text{Cl}$  by the  $\text{KI}$  in wash bottles, and the  $\text{CO}_2$  will be absorbed in the standard  $\text{Ba}(\text{OH})_2$  in Meyer tube.) Draw air thru apparatus 20 min., disconnect Meyer tube, and pour its contents into beaker. Wash out tube, adding washings to contents of beaker. Filter, wash, and titrate filtrate and washings with 0.1 *N*  $\text{HCl}$ , using phenolphthalein as indicator, 7.6(a). From number of ml of  $\text{Ba}(\text{OH})_2$  soln used and sp. gr. of sample, calculate percentage of  $\text{CO}_2$ . 1 ml of 0.1 *N*  $\text{Ba}(\text{OH})_2$  = 0.00220 g of  $\text{CO}_2$ .

## CALCIUM HYPOCHLORITE AND BLEACHING POWDER (43)

## AVAILABLE CHLORINE

## 6.146

*Arsenious Oxide Titration Method—Official*

Weigh 5–10 g of thoroly mixed sample into porcelain mortar, add 30–40 ml of  $\text{H}_2\text{O}$ , and triturate until smooth cream is obtained (high-test  $\text{Ca}(\text{OCl})_2$  will dissolve readily and not form a cream). Add more  $\text{H}_2\text{O}$ , stir well with pestle, and allow insoluble residue to settle few moments. Pour mixture off into liter volumetric flask, add more  $\text{H}_2\text{O}$ , and thoroly triturate sample and pour off as before. Repeat operation until all material has been transferred to flask. Rinse mortar and pestle, catch wash  $\text{H}_2\text{O}$  in flask, dilute soln to mark, and mix. Without allowing material to settle, pipet 25–50 ml aliquot into 200 ml Erlenmeyer flask. Add the standard  $\text{As}_2\text{O}_3$  soln, 6.139(a), in excess and then add a decided excess of  $\text{NaHCO}_3$ . Titrate excess of  $\text{As}_2\text{O}_3$  with the standard  $\text{I}$  soln, 6.3(b), using starch soln, 6.3(e), or the  $\text{I}$  as indicator. Subtract volume of  $\text{I}$  soln, corrected to 0.1 *N*, from volume of  $\text{As}_2\text{O}_3$  soln used, and calculate percentage of available  $\text{Cl}$ . 1 ml of 0.1 *N*  $\text{As}_2\text{O}_3$  = 0.003546 g of available  $\text{Cl}$ .

## CHLORAMINE T (43)

## ACTIVE CHLORINE

*Arsenious Oxide Titration Method—Official*

## 6.147

## REAGENTS.—See 6.139

## 6.148

## DETERMINATION

Transfer 0.5 g of sample to 300–500 ml Erlenmeyer flask, dissolve in 50 ml of  $\text{H}_2\text{O}$ , and add excess of the standard  $\text{As}_2\text{O}_3$  soln, 6.139(a), and 5 ml of  $\text{H}_2\text{SO}_4$  (1+4). Add decided excess of  $\text{NaHCO}_3$  and titrate excess  $\text{As}_2\text{O}_3$  with standard  $\text{I}$  soln, 6.3(b), using starch soln, 6.3(e), or the  $\text{I}$  as indicator. From this titration calculate active  $\text{Cl}$  in sample. 1 ml of 0.1 *N*  $\text{As}_2\text{O}_3$  soln = 0.001773 g of active  $\text{Cl}$ .

## 6.149

## TOTAL CHLORINE—OFFICIAL

Dissolve 0.5 g of sample in 50 ml of  $\text{H}_2\text{O}$  in Erlenmeyer flask and add slight excess of the standard  $\text{As}_2\text{O}_3$  soln calculated from the active  $\text{Cl}$  titration, 6.148. Add 5 ml of  $\text{HNO}_3$  (1+4), neutralize with  $\text{CaCO}_3$ , and titrate with standard  $\text{AgNO}_3$ , 6.91, using  $\text{K}_2\text{CrO}_4$ , 2.47(b), as indicator. Run blank titration on reagents and make correction for any  $\text{Cl}$  found. From corrected titration calculate percentage of total  $\text{Cl}$

in sample. 1 ml of 0.1 *N* AgNO<sub>3</sub> = 0.003546 g of Cl. If total Cl exceeds active Cl, presence of NaCl is indicated.

## 6.150

## SODIUM—OFFICIAL

Weigh 0.5 g of sample in silica or porcelain dish and add ca 25 ml of H<sub>2</sub>O and 3–5 ml of H<sub>2</sub>SO<sub>4</sub> (1+4). Evaporate to sirupy consistency on steam bath and finally to dryness on hot plate. Ignite at full heat of Bunsen burner, cool, and weigh as Na<sub>2</sub>SO<sub>4</sub>. (Residue should be completely soluble in H<sub>2</sub>O and should show no turbidity with NH<sub>4</sub>OH and (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>.) Test with flame for Na. If residue meets these tests it may be considered pure Na<sub>2</sub>SO<sub>4</sub>. From weight of residue calculate percentage of Na in sample.

## 2,2-BIS(p-CHLOROPHENYL)-1,1,1-TRICHLOROETHANE (DDT)

*Alcoholic Caustic Method—Tentative*

## 6.151

## REAGENTS

(a) *Standard silver nitrate soln.*—0.0282 *N*. Dissolve ca 4.791 g of analytical grade AgNO<sub>3</sub> in H<sub>2</sub>O and dilute to 1 liter. Standardize by titration against pure NaCl. 1 ml of 0.0282 *N* AgNO<sub>3</sub> soln = 1 mg of Cl.

(b) *Ammonium thiocyanate soln.*—0.0282 *N*. Dissolve ca 2.147 g of NH<sub>4</sub>CNS in H<sub>2</sub>O and dilute to 1 liter. Standardize by comparing with the standard AgNO<sub>3</sub> soln under the same conditions as obtain in determination.

## 6.152

## DETERMINATION

Place 50 ml of 0.1 *N* alcoholic KOH (Cl-free) in 500 ml flask fitted with reflux condenser. Transfer a portion of sample containing 25–200 mg of DDT into flask, either directly or by means of an aliquot of acetone soln. Reflux alcohol for 15 min. Add 100 ml of H<sub>2</sub>O and cool to room temp. Add 3 ml of HNO<sub>3</sub> and then standard AgNO<sub>3</sub> soln in excess of amount necessary to precipitate all the Cl. Add 0.5 g of Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> and 5 ml of nitrobenzene. Swirl flask to coagulate most of precipitate. Back-titrate excess of AgNO<sub>3</sub> with NH<sub>4</sub>CNS soln until faint pink color appears. Cross-titrate with both standard solns, crossing end point in each direction. (End point, which is not too sharp, is more easily perceived in this way.) Calculate percentage of DDT present on the basis that 1.00 mg of Cl = 10.00 mg of DDT.

## PHENOL COEFFICIENT (44)—OFFICIAL

## I. USING EBERTHELLA TYPHOSA

(Applicable to testing of coal tar disinfectants that are miscible with H<sub>2</sub>O and to other disinfectants that are miscible with H<sub>2</sub>O and act against bacteria in manner similar to phenol. False values are obtained from certain products that are highly inhibitory, such as Hg compounds, and the values obtained from testing oxidizing products may be highly misleading.)

## 6.153

## REAGENTS

(a) *Culture media.*—(1) *Nutrient broth:* Boil 5 g of beef extract (Difco), 5 g of NaCl, and 10 g of Armour's peptone (quality specially prepared for disinfectant testing) in 1000 ml of H<sub>2</sub>O 20 min., make to volume with H<sub>2</sub>O, and adjust to pH 6.8. (Using colorimetric method, adjust broth to give dark green color with bromothymol blue.) Filter thru paper, place 10 ml quantities in 20×150 mm bacteriological test tubes, plug with cotton, and sterilize at 15 lbs. pressure 40 min. Use

this broth for daily transfers and for subcultures. (2) *Nutrient agar*: Dissolve 1.5% Bacto agar (Difco) in nutrient broth and adjust to pH 7.2–7.4 (blue-green color with bromothymol blue), tube, plug with cotton, sterilize, and slant.

(b) *Test organism*.—The Hopkins strain of *Eberthella typhosa* (Zopf) Weldin (frequently called *Bac. typhosus*). Carry a stock culture on nutrient agar slants. Transfer once a month and incubate new stock transfer 2 days at 37°, then store at room temp.

From the stock culture inoculate a tube of nutrient broth and make at least 4 consecutive daily transfers (not over 30) in nutrient broth, incubating at 37°, before using culture for testing (if only 1 daily transfer has been missed it is not necessary to repeat the 4 consecutive transfers). Use 22–26 hours' culture of organism grown in nutrient broth at 37° in test. Shake, and allow to settle 15 min. before using.

(c) *Phenol* (45).—Use phenol that meets requirements of U.S.P. and has congealing point 40° or above. Use 5% soln as stock soln and keep in well-stoppered amber bottles in relatively cool place, protected from light. Standardize with 0.1 *N* K or Na bromide-bromate soln (46).

#### 6.154

#### APPARATUS

(a) *Glassware*.—1, 5, and 10 ml volumetric pipets; 1, 5, and 10 ml Mohr pipets graduated to 0.1 ml or less; 100 ml stoppered cylinders graduated in 1 ml divisions. Pyrex lipped test tubes 25 × 150 mm. Plug test tubes (medication tubes) with cotton wrapped in 1 layer of cheese-cloth. Sterilize all glassware 2 hours in hot air oven at 180°. Place pipets in closed metal containers before sterilizing.

(b) *Water bath*.—An insulated relatively deep water bath with cover having at least 10 well-spaced holes which admit medication tubes but not their lips.

(c) *Racks*.—May be of any convenient style. Blocks of wood (size depending somewhat on incubator to accommodate them) with deep holes are satisfactory. Have holes well spaced to insure quick manipulation of tubes; it is convenient to have them large enough to admit medication tubes while dilutions are being made.

(d) *Transfer loop*.—Make 4 mm (inside diam.) single loop at end of 2–3" Pt wire No. 23 B & S gage. Have other end in suitable holder (glass or Al rod). Bend loop at a 30° angle with stem (Fig. 11).

#### 6.155

#### PROCEDURE

Make 1% stock dilution of substance to be tested (or any other convenient dilution, depending on anticipated concn) in glass-stoppered cylinder. Make final dilutions, from the 1% stock dilution, directly into medication tubes and remove all excess over 5 ml. (Range of dilutions should cover killing limits of the disinfectant within 5 and 15 min. periods and should at the same time be sufficiently close for accuracy.) From the 5% stock soln make 1–90 and 1–100 dilutions of the phenol directly into medication tubes. Place these tubes, containing 5 ml each of final dilutions of disinfectant and of phenol, in water bath at 20° and leave 5 min. Add 0.5 ml of the test culture to each of the dilutions at time intervals corresponding to intervals at which transfers are to be made. (Thus, by the time 10 tubes have been seeded at 30 second intervals, 4.5 min. will have elapsed, and a 30 second interval intervenes before transference to sub-cultures is commenced.) Add culture from graduated pipet of sufficient capacity to seed all tubes in any one set. (As a precautionary measure the pipet should be loosely plugged with cotton at mouth end before being sterilized. Temp. of culture should be practically that of water bath before it is added.)

In inoculating medication tubes, hold them in slanting position after removal

from bath, insert pipet to just above surface of disinfectant, and run in culture without allowing tip to touch disinfectant. After adding culture, agitate tubes gently but thoroly to insure even distribution of bacteria, and replace in bath; 5 min. after seeding first medication tube, transfer 1 loopful of mixture of culture and diluted disinfectant from medication tube to corresponding sub-culture tube. To facilitate transfer of uniform drops of medication mixture, hold tube at 60° angle, and withdraw loop so that plane of loop is parallel with surface of liquid (see Fig. 11). At end of 30 seconds, transfer loopful from second medication tube to second sub-culture tube and continue process for each successive dilution; 5 min. after making first transfer begin second set of transfers for 10 min. period, and finally repeat for

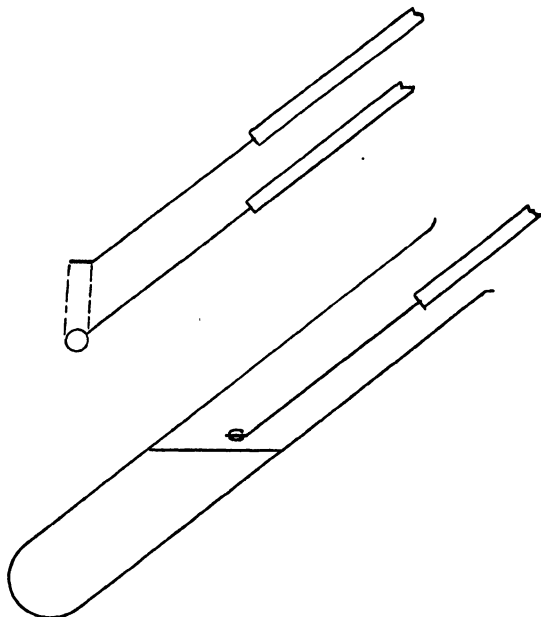


FIG. 11.—TRANSFER LOOP AND MANNER OF USING IN DETERMINATION OF PHENOL COEFFICIENT

15 min. period. Before each transfer heat loop to redness in Bunsen flame and flame mouth of every tube. Sterilize loop immediately after each transfer (before replugging tubes) to allow time for cooling. Use care in transferring and seeding to prevent pipet or needle from touching sides or mouth of medication tube and see that no cotton threads adhere to inner sides or mouths of tubes. Incubate sub-cultures at 37° for 48 hours and read results. Macroscopic examination is usually sufficient. Occasionally a 3-day incubation period, an agar streak, a microscopic examination, or agglutination with antityphoid serum may be necessary to determine feeble growth or suspected contamination.

#### 6.156

#### CALCULATION

Express results in terms of phenol coefficient, a number obtained by dividing numerical value of greatest dilution (denominator of fraction expressing dilution)

of the disinfectant capable of killing *Eb. typhosa* in 10 min. but not in 5 min. by greatest dilution of phenol showing same results.

*Example:*

Disinfectant (X):	5 MIN.	10 MIN.	15 MIN.
1-300	0	0	0
1-325	+	0	0
1-350	+	0	0
1-375	+	+	0
1-400	+	+	+
Phenol:			
1-90	+	0	0
1-100	+	+	+
Phenol coefficient would be $\frac{350}{90} = 3.89$ .			

The test is satisfactory only when phenol control gives one of following readings:

PHENOL	5 MIN.	10 MIN.	15 MIN.
1-90	+ or 0	+ or 0	0
1-100	+	+	+ or 0

If none of dilutions of disinfectant shows growth in 5 min. and killing in 10 min., estimate hypothetical dilution only when any 3 consecutive dilutions show following results: The first, no growth in 5 min.; the second, growth in 5 and 10 min. but not in 15 min.; and the third, growth in 5, 10, and 15 min.

*Example:*

Disinfectant (X):	5 MIN.	10 MIN.	15 MIN.
1-300	0	0	0
1-350	+	+	0
1-400	+	+	+
Phenol:			
1-90	0	0	0
1-100	+	+	0
Phenol coefficient would be $\frac{325}{95} = 3.42$ .			

To avoid giving an impression of fictitious accuracy, calculate phenol coefficient to nearest 0.1. Thus, in examples cited above, phenol coefficients would be reported as 3.9 and 3.4, instead of 3.89 and 3.42.

NOTE.—The commonly accepted criterion that disinfectants for general use be employed at a dilution equivalent in germicidal efficiency to 5% phenol against *Eb. typhosa* (that is, 20 times the *Eb. typhosa* coefficient) allows reasonable margin of safety for destruction of infective agents likely to be the object of general disinfection.

## 6.157

### II. USING STAPHYLOCOCCUS AUREUS (44)

Proceed as directed in 6.153–6.156, except to change the phenol dilutions and test organisms. Use temp. of 20° unless otherwise directed. The culture of *Staph. aureus* must have at least the resistance indicated by the following:

AT 20°	PHENOL	5 MIN.	10 MIN.	15 MIN.
	1-60	+	0	0
	1-70	+	+	+

The resistance of the culture to phenol when used at 37° must be as follows:

PHENOL	5 MIN.	10 MIN.	15 MIN.
1-80	+	0	0
1-90	+	+	+ or 0

### FUNGICIDAL TEST (47)

#### USING TRICHOPHYTON INTERDIGITALE—TENTATIVE

##### 6.158

##### TEST ORGANISM

Use as the test fungus a typical strain of *Trichophyton interdigitale* isolated from dermatophytosis of the foot. The strain must sporulate freely on artificial media, the presence of abundant conidia being manifested by powdery appearance on surface of 10-day culture, particularly at top of an agar slant, and confirmed by microscopic examination. The conidia-bearing mycelium should peel easily from surface of dextrose agar. Conidia of required resistance survive a 10 min. exposure at 20° to a phenol dilution of 1:60, but not to one of 1:45. A suitable strain (No. 640) has been deposited with the American Type Collection.

##### 6.159

##### CULTURE MEDIUM

Carry the fungus on agar slants of following composition: C.P. dextrose 2%, Neopeptone (Difco) 1%, agar 2%, adjusted to pH 5.6-5.8. Use same culture medium in preparing cultures for obtaining a conidial suspension; and use a fluid medium of the same nutrient composition (without agar) to test the viability of conidia after exposure to fungicide.

##### 6.160

##### CARE OF FUNGUS STRAIN

Store stock culture of the fungus on dextrose agar slants at 2-5°. At intervals not to exceed 3 months transfer it to fresh agar slants, incubate at 25-30° for 10 days, and then place in storage at 2-5° until next transfer period. Do not use culture that has been kept at room or higher temp. for more than 10 days as source of inoculum for culture purposes. (Cultures may be kept at room temp. for preservation of the strain and for the inoculation of cultures if transferred at intervals not exceeding 10 days.)

##### 6.161

##### PREPARATION OF CONIDIAL SUSPENSION

Prepare Petri dish agar cultures by planting the inoculum at center of agar plate and by incubating culture at 25-30° for 10, and not exceeding 15, days. Then remove the mycelial mat from agar surface of each plate by means of sterile spatula or heavy flattened wire and suspend in 20-25 ml of physiological NaCl soln (0.85% NaCl) per plate. Free the conidia from the mycelium by shaking mixture gently with or without glass beads, and filter suspension so that hyphal elements are removed while conidia pass thru. (Sterile absorbent cotton in sterile funnel is suitable. This should yield 10-25 million conidia/ml.) Estimate density of conidial suspension by counting in a haemocytometer and dilute with physiological NaCl soln so that it contains 5 million conidia/ml.

##### 6.162

##### OPERATING TECHNIC

Prepare dilutions of the fungicide. Test procedures are similar to those outlined in 6.155. Place 5 ml portions of each fungicide soln and the phenol control solns in 25×150 mm test-culture tubes, arrange in order of ascending dilutions, and place these tubes in a 20° water bath until temp. of bath is reached. With graduated

pipet place 0.5 ml of the spore suspension in first tube of fungicidal soln, shake tube, and immediately replace in water bath; 30 seconds later add 0.5 ml of the conidial suspension to second tube. Repeat this procedure at 30 second intervals for each fungicidal dilution. If more convenient run the test at 20 second intervals. After 5, 10, and 15 minute periods of exposure to the fungicide remove a sample from each conidia-fungicide mixture with a 4 mm loop and place in 10 ml of the dextrose broth. To eliminate risk of faulty results due to possibility of fungistatic action make sub-transfers by withdrawing a loopful of the conidia-fungicide mixture, touching it to surface in tube of dextrose broth as described above, and immediately immersing it in second tube of broth. Incubate the inoculated tubes at 25–30°. Read final results after 10 days, altho an indicative reading can be made in 4 days.

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## 7. CAUSTIC POISONS

### PREPARATIONS CONTAINING PHENOL

#### PHENOL

##### *Method I(1)—Official*

(Applicable to determination of phenol in commercial cresols, saponified cresol solns, coal tar dips, and disinfectants, and to kerosene solns of phenols except in presence of salicylates or betanaphthol.)

#### 7.1

##### REAGENTS

(a) *Dilute nitric acid*.—Blow air thru  $\text{HNO}_3$  until it is colorless and dilute 1 volume of this acid with 4 volumes of  $\text{H}_2\text{O}$ .

(b) *Millon reagent*.—Treat 2 ml of Hg in 200 ml Erlenmeyer flask with 20 ml of  $\text{HNO}_3$ . Place flask under hood, and after first violent reaction is over shake as much as necessary to effect subdivision of Hg and maintain action. After ca 10 min., when action has practically ceased even in presence of undissolved Hg, add 35 ml of  $\text{H}_2\text{O}$ , and if basic salt separates, add sufficient quantity of the dilute  $\text{HNO}_3$  to dissolve it. Add 10% NaOH soln dropwise with thoro mixing until the curdy precipitate that forms after the addition of each drop no longer redissolves but disperses to an evidently permanent turbidity. Add 5 ml of the dilute  $\text{HNO}_3$  and mix well. As the soln deteriorates, do not use it after the first day.

(c) *Standard phenol soln*.—Dissolve weighed quantity of the pure substance (congealing point not lower than  $40^\circ$ ) in sufficient  $\text{H}_2\text{O}$  to make not less than 1% soln. On day it is to be used, from this stock soln make a 0.025% soln (final standard) in additional  $\text{H}_2\text{O}$ .

(d) *Formaldehyde soln*.—Dilute 2 ml of commercial 37% HCHO soln to 100 ml with  $\text{H}_2\text{O}$ .

#### 7.2

##### APPARATUS

(a) *Nessler cylinders*.—50 ml tall form, matched.

(b) *Test tubes*.—Approximately  $180 \times 20$  mm, provided with rubber stoppers and marked at 25 ml.

(c) *Water bath for heating test tubes*.—A beaker containing disk of wire gauze raised about an inch from bottom may be used.

#### 7.3

##### PREPARATION OF SAMPLE

(a) *Commercial cresol*.—Weigh by difference ca 2.5 g of sample into 250 ml volumetric flask, dissolve in 10 ml of 10% NaOH soln, and make to mark with  $\text{H}_2\text{O}$ .

(b) *Saponified cresol solns, coal tar dips and disinfectants, kerosene solns of phenols, etc*.—Weigh by difference ca 5 g (or use 5 ml and calculate weight from density) of sample into 250 ml volumetric flask and dilute to mark with  $\text{H}_2\text{O}$ . In products consisting largely of kerosene, bring  $\text{H}_2\text{O}$  level to mark and take aliquots from aqueous portion only.

#### 7.4

##### DETERMINATION

Transfer 5 ml aliquot of prepared soln to 200 ml volumetric flask shortly before determination is to be carried out, dilute to ca 50 ml, add 1 drop of methyl orange indicator, 6.3(f), and then the dilute  $\text{HNO}_3$  until soln is practically neutral, make to volume, and shake well.

Place 5 ml of the diluted soln in each of 2 of the marked test tubes, and in each of 2 additional test tubes place 5 ml of the standard phenol soln. Next flow 5 ml of the Millon reagent down side of each tube, mix, and place tubes in bath of boiling  $H_2O$ ; continue boiling exactly 30 min., cool immediately and thoroly by immersion in bath of cold  $H_2O$  at least 10 min., and add 5 ml of the dilute  $HNO_3$  to each tube.

Mix well and add 3 ml of the dilute  $HCHO$  soln to one of each pair of tubes. Make all tubes to 25 ml mark with  $H_2O$ , stopper, shake well, and allow to stand overnight. The next day the contents of tubes to which  $HCHO$  was added will have faded to yellow, while the others will show orange or red tint.

Pipet 20 ml from each of the 2 phenol tubes and transfer to 100 ml volumetric flasks; treat each with 5 ml of the dilute  $HNO_3$ , make to mark, and mix. The red flask contains the "phenol standard," and the yellow flask the "phenol blank." Transfer these solns to burets. Pipet 10 ml of each sample soln into Nessler tubes. (The orange or red constitutes the "unknown" and the yellow the "sample blank," and each Nessler tube must be distinctly marked to avoid confusion.) Add to "sample blank" tube measured quantity of "phenol standard" and add same volume of "phenol blank" to "unknown," thoroly agitate (aided by insertion of rubber stoppers, if necessary), and compare colors. When tubes have been brought to match, each ml of phenol standard used = 1% of phenol if portion of sample weighing exactly 5 g was used, or 2% if exactly 2.5 g was used.

**NOTE.**—In using this method the following precautions should be borne in mind: A pair of phenol tubes affords sufficient final solns for assaying several unknowns, but all the latter must have accompanied phenol solns thruout entire process with identical reagents and treatment. If end point has been inadvertently overrun it is possible to work back to it, but since mistakes are easy to make in this procedure it is better to repeat comparison on fresh portions from original tubes. Too much delay in matching tubes must be avoided after titration has been started, otherwise excess of  $HCHO$  present in blanks may have time after mixture to affect intensity of red color.

Millon reagent is dangerously poisonous and should not be transferred with an ordinary pipet and mouth suction unless a protective trap of some kind is used.

## 7.5

*Method II (2)—Official*

(Applicable to determination of phenol in presence of salicylates.)

Weigh by difference into a separator 10 g of sample (or use 10 ml and calculate weight from density of sample). Add 50 ml of kerosene and extract 3 times with 100 ml portions of  $H_2O$ . Filter aqueous extracts thru wet filter into 500 ml volumetric flask, make to volume with  $H_2O$ , and proceed as directed under 7.4.

When tubes have been brought to match, each ml of the phenol standard used = 1% of phenol if a portion of sample weighing exactly 10 g was used.

## SODA LYE

## CARBONATE AND HYDROXIDE (3)—OFFICIAL

## 7.6

## REAGENTS

(a) *Phenolphthalein indicator.*—Dissolve 1 g of phenolphthalein in 100 ml of neutralized alcohol.

(b) *Barium chloride soln.*—Dissolve 100 g of  $BaCl_2 \cdot 2H_2O$  and dilute to 1 liter.

## 7.7

## DETERMINATION

Weigh ca 10 g of sample from weighing bottle, dissolve in  $CO_2$ -free  $H_2O$ , and dilute to definite volume. Titrate aliquot of this soln with the 0.5 N  $HCl$ , 43.7–43.8,

using the methyl orange indicator, 6.3(f), and note the total alkalinity thus found. Transfer an equal aliquot to volumetric flask and add enough of the  $\text{BaCl}_2$  soln to precipitate all the carbonate, avoiding any unnecessary excess. Dilute to mark with  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ , stopper, shake, and set aside. When liquid becomes clear, pipet off one-half and titrate with the 0.5 *N*  $\text{HCl}$ , using the phenolphthalein indicator; ml of 0.5 *N* acid required for this titration  $\times 2$  = ml of 0.5 *N* acid equivalent to  $\text{NaOH}$  present in original aliquot. Difference between this figure and the ml of 0.5 *N*  $\text{HCl}$  required for total alkalinity represents the ml of 0.5 *N* acid equivalent to the  $\text{Na}_2\text{CO}_3$  present in aliquot. Calculate percentages of  $\text{Na}_2\text{CO}_3$  and  $\text{NaOH}$ .

## SELECTED REFERENCES

- (1) U. S. Dept. Agr. Bull. 1308, p. 17; J. Assoc. Official Agr. Chem., 13, 160 (1930).
- (2) Ind. Eng. Chem., Anal. Ed., 1, 232 (1929).
- (3) Sutton, "Systematic Handbook of Volumetric Analysis," 10th ed., p. 61 (1911).

## 8. NAVAL STORES

### ROSIN

#### 8.1

##### SAMPLING—OFFICIAL

By means of large steel spike break and remove part of top rosin in barrel or drum to depth of 3–4". Take 1 or 2 large lumps from below such depth, with total wt. at least 1 lb. If rosin is in small packages (100 lb. bags) take  $\frac{1}{2}$  lb. lump from each of 4 bags of each grade or mark. Do not break up or pulverize lumps. For acid number, saponification number, or other analytical tests, obtain stated quantity of sample by breaking off small pieces having freshly exposed surfaces, rejecting small particles (necessary because rosin oxidizes when exposed to air in finely divided form).

#### 8.2

##### ACID NUMBER—OFFICIAL

Weigh 2 g of the rosin into 250–300 ml Erlenmeyer flask, add 50 ml of neutral alcohol (denatured, Bur. Int. Rev. Formula 30 is suitable—1 vol. methyl to 10 vols. 95% ethyl alcohol). Warm to dissolve and titrate at once with 0.5 *N* NaOH soln, using 1 ml of phenolphthalein soln as indicator. Add alkali rapidly and when end point is approached (after 10 ml added) add 30 ml of hot H<sub>2</sub>O. Continue titration with vigorous shaking and rapid additions of NaOH soln to 30 second permanent pink end point. (For lighter colored rosins, H<sub>2</sub>O may be omitted.)

For dark rosins, the end point may be more readily determined by observing development of absorption band in spectrum of white light passed thru a 1" layer of the soln (1), using pocket spectroscope and holding flask in inclined position in front of light source (preferably daylight). The pink phenolphthalein salt absorbs light in green part of spectrum between 546 and 577 m $\mu$ , causing faintly visible absorption band or dark area in that part of spectrum. Preliminary observation should be made on blank soln containing 100 ml of alcohol, 1 ml of indicator soln, and 2 drops of NaOH soln.

#### 8.3

##### SAPONIFICATION NUMBER—OFFICIAL

Weigh 2.5 g of rosin sample into 250–300 ml alkali-resistant Erlenmeyer flask. Do not use etched flasks, as glass will react with the alkali. Add 25 ml of alcoholic KOH soln, 31.24, allowing pipet to drain for definite time. Connect flask to reflux condenser, place on hot plate, and boil vigorously for exactly 1 hour from time boiling starts. Cool, and titrate with 0.5 *N* HCl, using 1 ml of phenolphthalein soln as indicator. Conduct two blank determinations, using same pipet for measuring KOH soln and draining for same length of time. Subtract ml of 0.5 *N* HCl used in determination on sample from ml of HCl used for blank titration, to obtain ml equivalent to KOH used for saponification of sample. Calculate as saponification number the mg of KOH required to saponify 1 g of the rosin. Dilute soln if necessary with neutral alcohol to obtain sharp end point. If dark color of soln prevents observation of end point, use spectroscopic method, 8.2. End point is reached when addition of 1–2 more drops of the acid causes disappearance of characteristic absorption band. Similar quantity of 0.5 *N* NaOH should bring band back.

#### 8.4

##### UNSAAPONIFIABLE MATTER—TENTATIVE

Dissolve 30 g of rosin in 200 ml of alcohol (Formula 30, 8.2, is satisfactory) in 500 ml round-bottomed flask, warming gently until dissolved. Cool slightly, add 6 ml of 50% NaOH soln (sp. gr. 1.52), connect to reflux condenser, and boil gently 1.5 hours. Add 150 ml of H<sub>2</sub>O and ebullition tube, attach to regular condenser, and

place flask in oil bath held at 120–130°. Distil off 210 ml of distillate. Quantitatively transfer residual soap soln from flask into 250 ml volumetric flask, and make to mark with  $H_2O$ . Pipet 50 ml of aliquot of thoroly mixed soap soln into an extractor tube (Fig. 12) (§) and add 100 ml of ether to 250 ml flask. Attach vertical condenser to top of extractor tube and place flask in hot water bath maintained at 50–55° (hot plate). Extract for 6 hours refluxing at ca 3 drops/second. Transfer ether extract to

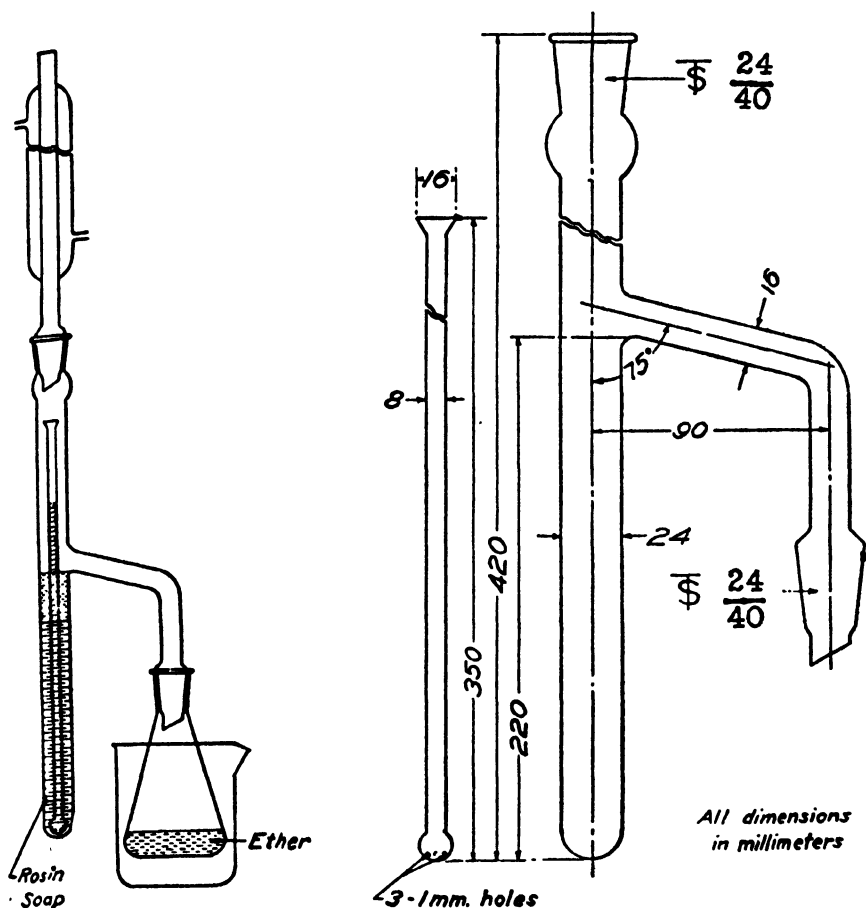


FIG. 12.—UNSAAPONIFIABLE EXTRACTION APPARATUS—COMPLETE ASSEMBLY AND DETAILS OF EXTRACTOR TUBE

500 ml separator, rinsing flask several times with 15 ml of ether. Wash extract with 50 ml of 1% NaOH soln, draw off alkali into second separator, extract this with 30 ml fresh ether, draw off aqueous portion, and combine ether extracts. Wash 3 times with 50 ml of  $H_2O$ . Run washed ether extract into tared 250 ml flask and distil off ether, immersing flask in water bath at 50–60°. Finally raise bath temp. to 70° and connect flask to vacuum line at 20–25 mm Hg for 30 min. Cool, and

weigh flask to obtain "net washed extract" from 6 g of rosin sample. Dissolve residue in flask in 40 ml of neutral alcohol and titrate with 0.1 *N* KOH, using phenolphthalein indicator. KOH soln used represents free rosin acid in extract.

$$\text{Per cent rosin acid} = \frac{\text{ml KOH} \times N \times 30.2}{\text{wt. rosin (6 g)}}.$$

Per cent net washed extract—per cent rosin acid—per cent unsaponifiable matter.

### 8.5 PETROLEUM BENZINE—INSOLUBLE MATERIAL (OXIDIZED ROSIN)—OFFICIAL

(This method determines degree of oxidation or decomposition (pitch formation) of rosin. If appreciable quantity of dirt or other extraneous foreign matter is present, proceed according to 8.6 and subtract toluene-insoluble matter from total quantity of petroleum benzine-insoluble matter.)

Weigh 1 g of freshly ground rosin into tared 250 ml glass-stoppered (cork covered with tin foil may be used) Erlenmeyer flask. Add 100 ml of petroleum benzine (b.p. 30–75°), stopper, and shake to prevent rosin coalescing and adhering to glass. Add additional 50 ml of petroleum benzine, stopper, and shake until any undissolved rosin is in finely divided state and does not adhere to flask. Allow to stand overnight at room temp., mix contents with whirling motion, and filter thru tared Gooch crucible. Rinse flask twice with 25 ml portions of petroleum benzine, passing washings thru crucible. Wipe outside of flask and crucible with cloth dampened with alcohol or acetone, and dry in oven at 95–100° for 2 hours. Cool, and weigh. From combined weight of residues in flask and crucible calculate percentage of petroleum benzine-insoluble matter.

### TOLUENE—INSOLUBLE MATERIAL (3)—OFFICIAL

#### 8.6

#### PREPARATION OF SAMPLE

(Dirt or extraneous foreign matter may interfere with the adaptability of rosin for some purposes. This method provides a measure of the foreign matter (dirt, sand, chips, bark) in rosin.)

Shortly before making determination take pieces from all lumps comprising a sample (cubes for grading may be used for this test) to give ca 200 g. Crush to pass  $\frac{1}{2}$ " sieve, mix, grind to pass No. 10 sieve, again mix, and fill a small wide-mouthed bottle. Keep stoppered until used.

#### 8.7

#### DETERMINATION

Place 50 g of ground rosin in 300 ml beaker, add 150 ml of toluene (free from non-volatile residue), and dissolve sample with aid of heat and occasional shaking. When no more undissolved rosin remains, filter thru 25 ml porcelain Gooch crucible that has been previously prepared with mat of pure, well-washed asbestos, washed thoroly with toluene, dried at 105–110° for 30 min., cooled in desiccator, and weighed. If rosin filtrate is not clear, return thru Gooch crucible until clear, finally washing residue free from rosin with additional warm solvent. Wipe off outside of crucible with cloth dampened with solvent. Dry to constant weight at 105° (1 hour usually suffices), cool in desiccator, weigh, and calculate percentage of toluene-insoluble solid matter in the rosin.

#### 8.8

#### ASH—OFFICIAL

Weigh 10 g of rosin in 50 ml porcelain crucible or Vycor evaporating dish. Warm to melt sample, ignite, and burn off combustible matter (hood). Ignite residue until ash is free from carbonaceous matter (1 hour in muffle furnace at 1400°), cool, and weigh. Report as percentage of ash.

**8.9 VOLATILE OILS (TURPENTINE OR MINERAL OIL)—TENTATIVE**

Place 50 g of freshly broken rosin in 300–500 ml round-bottomed flask, add 100 ml of glycerol and 25 ml of  $H_2O$ . Mix contents and connect to light oil separatory trap fitted with stopcock (Chap. 33, Fig. 56). Attach water tube condenser, and partly fill trap with  $H_2O$ . Insert thermometer into liquid in flask thru neck, with bulb near bottom, or use flask with thermometer well. Bring contents of flask to gentle boil, collecting  $H_2O$  and oil in trap. Remove  $H_2O$  from system by drawing off 5 ml portions from time to time, to raise boiling temp. of  $H_2O$ -glycerol mixture. Continue distillation until temp. reaches 180–190° (2 hours), and remove heat. Read volume of oil collected, and report as ml/100 g of rosin. To calculate percentage by weight, use 0.875 for density of oil. To determine nature of the oil, allow to stand until clear, separate from  $H_2O$ , read refractive index, and run polymerization test, 8.19.

**TURPENTINE OIL (SPIRITS OF TURPENTINE)****COLOR GRADE****8.10***Lovibond Glass Method—Official*

Fill flat-bottomed colorimeter tube (preferably graduated at each 2 mm) to depth of 40–50 mm with turpentine to be tested, and place over or under it (in colorimeter if available) a No. 2 Yellow Lovibond Glass. Over or under a second tube place a No. 1 Yellow Lovibond Glass, and pour turpentine into this tube until total color of turpentine plus glass matches as nearly as possible the color of other tube plus glass. Difference in depth of columns of turpentine is equivalent to color of the No. 1 glass. If this difference is 150 mm or more, the turpentine is "waterwhite"; if 50–149 mm, it is "standard"; if 25–49 mm, it is "one shade off."

**8.11***Potassium Dichromate Method (4)—Tentative*

Compare color of turpentine contained in colorimeter tube, cell, or 4 oz. oil sample bottle with color of equal depth (at least 100 mm) of freshly prepared solns containing  $K_2Cr_2O_7$  and  $CoCl_2$ , of following concentrations per liter: for "waterwhite"—0.0072 g  $K_2Cr_2O_7$  + 0.07 g  $CoCl_2 \cdot 6H_2O$ ; for "standard"—0.0180 g  $K_2Cr_2O_7$  + 0.15 g  $CoCl_2 \cdot 6H_2O$ .

Since such dilute solns are unstable in glass, for frequent tests the required solns may be prepared from stock solns made up as follows: No. 1—1.80 g  $K_2Cr_2O_7$  + 1 ml  $HCl$ /1000 ml; No. 2—1.00 g  $CoCl_2 \cdot 6H_2O$  + 20 ml  $HCl$ /1000 ml. The test solns are prepared from 1 and 2 as follows: "Waterwhite"—2 ml No. 1 + 35 ml No. 2 diluted to 500 ml; "standard"—5 ml No. 1 + 75 ml No. 2 diluted to 500 ml.

**8.12****SPECIFIC GRAVITY—OFFICIAL**

Determine sp. gr. at 15.5/15.5° by any convenient method having a precision of 5 points in fourth place. If apparatus standardized at 15.5° is used, reading may be taken at any convenient temp. and corrected to 15.5° by adding thereto or subtracting therefrom 0.00082 for each degree that temp. at which determination is made is respectively above or below 15.5°.

**8.13****REFRACTIVE INDEX—OFFICIAL**

Determine refractive index at any convenient temp., preferably at 20°. If determined at other than 20°, calculate result to 20° by adding or subtracting the correction factor 0.00045 for each degree that temp. of determination is above or below 20°.



## DISTILLATION—OFFICIAL

## 8.14

## APPARATUS

(a) *Flask*.—Engler flask with following dimensions: diam. of bulb 6.5 cm; neck 15 cm long, 1.6 cm internal diam.; side or vapor tube 10 cm long, 0.6 cm external diam., attached to neck at angle of 75°; distance from surface of liquid (100 ml) to bottom of junction of side tube and neck, 9.0 cm.

(b) *Support for flask*.—Asbestos plate  $\frac{1}{2}$ " thick on movable ring; plate ca 20 cm square (to fit snugly in shield), having opening 3.5–4 cm in diam. in center.

(c) *Shield*.—Surround flask and support (Fig. 13) with metal shield having hinged opening in front, ca 8" square, and 17–19" high (higher shield is preferable, to keep air currents off neck of flask).

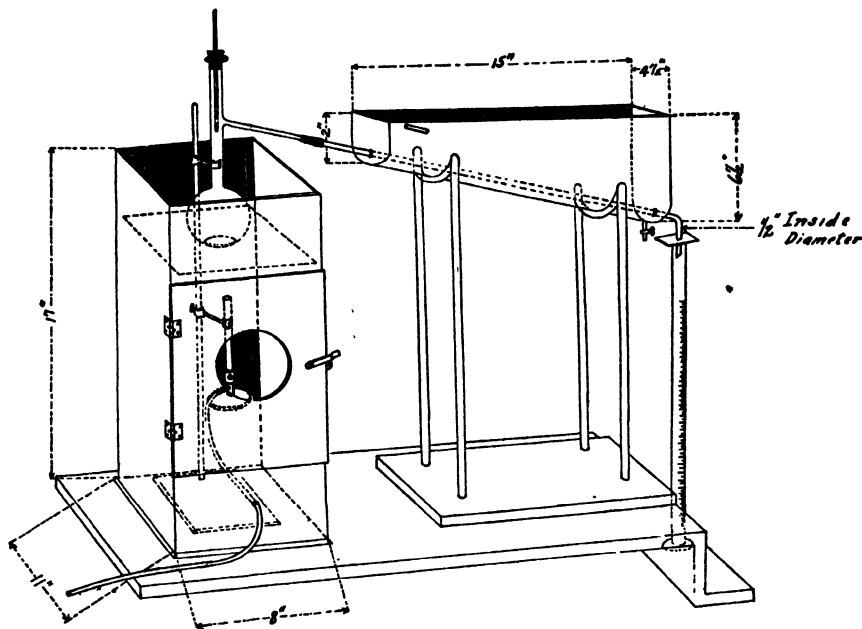


FIG. 13.—APPARATUS FOR DISTILLATION OF TURPENTINE OIL  
OVER OPEN FLAME

(d) *Condenser*.—(1) Use form illustrated in Fig. 13, which consists of thin-walled brass condenser tubing (No. 20 Stubbs gage seamless)  $\frac{1}{2}$ " inside diam. and 22" long, placed at angle in metal cooling bath, as shown, with lower end of tube cut off at acute angle and curved down for length of ca 3", to project into receiving cylinder; or, (2) use straight Liebig glass condenser 22" long, with 16" in contact with cooling  $H_2O$  and fitted with adapter to extend short distance below top of receiving cylinder.

(e) *Thermometer*.—N-filled mercurial thermometer scaled for partial immersion, conforming to following specifications: Immersion 76 mm; range 147–182°, in 0.5° intervals; length over all ca 295 mm; bottom of bulb to 147° mark 100–115 mm; stem diam. 6–7 mm; bulb diam. 4–5.5 mm; bulb length 10–15 mm; maximum error at any point 0.5°. (These specifications describe the ASTM Turpentine Distillation

Thermometer, ASTM Designation E1-27C; the ASTM Solvents Distillation Thermometer, ASTM Designation E1-42C, or an Anschütz type, total immersion thermometer, graduated from 145° to 200°, may be used.)

(f) *Receiving cylinder.*—For general purposes, use ordinary 100 ml graduated cylinder marked in 1 ml subdivisions. For determining quantity distilling to specified temp. below upper limit, 50 ml precision cylinders graduated in 0.2 ml are preferred. Cover top of cylinder with pasteboard cover having opening for condenser tube.

**8.15****DETERMINATION**

Using receiving cylinder, carefully transfer 100 ml of turpentine into bulb of flask, allowing cylinder to drain. Add several pieces of pumice or glass. Fit cork with thermometer so top of Hg bulb (or top of contraction chamber if ASTM Solvents Distillation Thermometer is used) is opposite bottom of side tube. Place flask securely in position on asbestos board and connect with condenser. Apply heat carefully at first, regulating so that first drop falls from condenser in 5–10 min., and distillation then proceeds at rate of 4–5 ml/min. (ca 2 drops/second). Initial boiling point is thermometer reading (corrected) when first drop falls from end of condenser. Remove heat and discontinue distillation when temp. reaches 170° (180° in case of destructively distilled wood turpentine) or the corrected equivalent thereof, depending on atmospheric pressure, as determined under 8.16. Let condenser drain and read percentage distilled. (If percentage distilled below intermediate temp. is recorded, apply similar atmospheric pressure correction to such temp.)

**8.16****CORRECTION FOR VARIATION IN ATMOSPHERIC PRESSURE**

As distilling temp. of turpentine and similar terpene hydrocarbons varies by 0.057° for each mm change in barometric pressure, for concordant results correct the observed initial boiling point and distillation temperature limits to standard atmospheric conditions, i.e., 760 mm Hg at 0°. Note barometer reading and temp. of air at barometer. From table, 8.17, interpolating and using temp. column most nearly corresponding to observed air temp., compute temp. correction to nearest 0.1° to be applied to observed initial boiling point. Apply similar correction (changing the + or – sign) to temp. at which distillation is stopped.

**EXAMPLE:** Barometer reads 748 mm at 22° air temp. Interpolating between corrections in 20° column for 740 and 750 mm, correction for 748 mm is +0.82°. Therefore initial boiling point is corrected by +0.8° (added to observed reading) and likewise, final distilling temp. must be corrected by –0.8° (subtracted from prescribed limit), i.e., 169.2° instead of 170°.

**8.17 Turpentine distilling temperature correction for various barometric pressures\***

OBSERVED BAROMETRIC PRESSURE		TEMPERATURE CORRECTION FOR BAROMETER TEMPERATURE OF—			
mm	in.	80°	85°	90°	95°
780	30.7	–1.0°	–0.96°	–0.92°	–0.88°
770	30.3	–0.43	–0.39	–0.36	–0.32
760	29.9	+0.14	+0.18	+0.21	+0.25
750	29.5	+0.71	+0.74	+0.78	+0.82
740	29.1	+1.26	+1.30	+1.34	+1.38
730	28.7	+1.84	+1.87	+1.91	+1.95
720	28.3	+2.41	+2.45	+2.48	+2.52
710	28.0	+2.98	+3.01	+3.05	+3.09
700	27.6	+3.55	+3.58	+3.61	+3.65

\* These figures are based on value of 0.057°/mm (Landolt-Börnstein Tabellen, Ed. 4, p. 436) together with corrections (from Circular F, Instrument Div., U. S. Weather Bureau) that must be applied to observed barometer reading to allow for contraction of the Hg, glass tube, and brass scale if barometer were cooled to 0°.

## MINERAL OIL IN TURPENTINE

*Polymerization with Fuming Sulfuric Acid (4)—Official*

## 8.18

## REAGENT

*Fuming 38N sulfuric acid.*—In tared glass-stoppered bottle (usual 2½ liter acid bottle is convenient) mix fuming  $\text{H}_2\text{SO}_4$  (free from N oxides) (A) with  $\text{H}_2\text{SO}_4$  (B) to obtain mixed acid (C), containing slightly more than 82.38% total  $\text{SO}_3$ . Depending on strength of fuming acid available, use following proportions of two acids:

- 100 parts A (15–20% free  $\text{SO}_3$ ) to 50 parts B;
- 100 parts A (20–30% free  $\text{SO}_3$ ) to 75 parts B; and
- 100 parts A (50% free  $\text{SO}_3$ ) to 140 parts B.

After thoro mixing (considerable heat is generated), allow to cool, and again weigh to determine quantity of mixed acid obtained. Ascertain exact strength of mixed acid (C) and also of reserve supply of acid (B) by following procedure:

Pour ca 50 ml into small beaker and fill weighing bulb or pipet of ca 10 ml capacity, by slight suction, wiping off outside of bulb with moist, then with dry cloth. Weigh on analytical balance and allow acid to flow slowly down sides of neck of 1000 ml volumetric flask into ca 200 ml of cold  $\text{H}_2\text{O}$ . (This relation between size of bulb and flask gives resultant soln near 0.5 N.) When bulb has drained, wash all traces of acid into flask, taking precautions against loss of  $\text{SO}_3$  fumes. Make to volume and titrate from buret with standard alkali, using same indicator as used in standardizing. Calculate  $\text{SO}_3$  content of both acids, and add calculated quantity of reserve acid (B) to quantity of mixed acid (C) on hand to bring C to 82.38% total  $\text{SO}_3$  (equivalent to 100.92%  $\text{H}_2\text{SO}_4$ ). Permissible variation is  $\pm 0.15\%$   $\text{H}_2\text{SO}_4$ . After adding required quantity of B, again analyze mixed acid to make certain it is of proper strength, finally running test on a turpentine sample known to be pure. Keep acid in small bottles or in special dispenser bottle (5) to prevent absorption of moisture from air.

## 8.19

## DETERMINATION

Place 20 ml of the 38 N  $\text{H}_2\text{SO}_4$  in narrow-necked Babcock bottle, stopper, and set in ice water to cool. Add slowly from pipet, a little at a time, 5 ml of the turpentine, leaving bottle unstoppered, and gently shaking or rotating flask, keeping cool by repeated immersion in ice water. When all of sample is delivered to bottle, and mixture no longer develops excessive heat on shaking, shake vigorously ca ½ min. Place bottle in water bath held at 60–65° and heat for 10 min., with intermittent vigorous shaking not less than 6 times during heating period. (CAUTION: Hold bottle in slightly inclined position, and let first few shakes be gentle, to allow released  $\text{SO}_3$  to escape without forcing some of liquid up over mouth of bottle.) Cool to room temp. and fill with ordinary  $\text{H}_2\text{SO}_4$  until surface rises well into graduated neck. Centrifuge 5 min. at 1200 r.p.m. or 10 min. at 900 r.p.m., or allow to stand, lightly stoppered, overnight. Read volume of unpolymerized residue (middle of meniscus), calculate percentage, record consistency and color, and determine its refractive index at 20°.

By this method a pure turpentine gives less than 2.0% residue of a straw or darker color and viscous consistency, with refractive index of 1.500 or more. A limpid, colorless residue with refractive index below 1.500 indicates presence of mineral oil. The unpolymerized residue from an adulterated oil represents 60–80% of total quantity of adulterant present.

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- (1) Ind. Eng. Chem., Anal. Ed., **6**, 122 (1934).
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- (3) J. Assoc. Official Agr. Chem., **13**, 48 (1930).
- (4) U. S. Dept. Agr., Bur. Chem. Circ. **85**; J. Assoc. Official Agr. Chem., **6**, 465 (1923).
- (5) Ibid.; also U. S. Dept. Agr., Bur. Chem. Bull. **898**.

## 9. GELATIN, DESSERT PREPARATIONS, AND MIXES

### GELATIN—TENTATIVE

9.1

#### PREPARATION OF SAMPLE

In the case of ground gelatin mix thoroly. In the case of sheet gelatin break sheets into small pieces by hand. Further comminution is unnecessary in either case.

9.2

#### MOISTURE

Proceed as directed under 20.3, using 2 g of material prepared as directed under 9.1.

9.3

ASH.—See 34.9 or 34.10

9.4

#### TOTAL PHOSPHORUS

Treat the ash, 9.3, with 2–3 ml of  $\text{HNO}_3$  and evaporate to dryness on steam bath. Repeat  $\text{HNO}_3$  treatment and evaporation, take up residue in hot  $\text{H}_2\text{O}$  containing few drops of  $\text{HNO}_3$ , and proceed as directed under 2.9.

9.5

#### NITROGEN

Proceed as directed under 2.24, 2.25, or 2.26, using 1 g of sample. To convert to gelatin multiply by factor 5.55 (1).

9.6

#### JELLY STRENGTH

To 7.50 g of sample add from pipet, with stirring, 100 ml of  $\text{H}_2\text{O}$  at 10–15°. Let stand 30 min. and then bring to 45° in water bath; remove from bath and let stand 15 min. Mix by inversion, let stand 15 min., place in water bath controlled at 10°  $\pm 0.1^\circ$ , and chill for 17 hours. Determine jelly strength on Bloom Gelometer (2), adjusted to deliver 200 g of shot/5 seconds ( $\pm 5$  g), using the  $\frac{1}{4}$ " plummet.

### GELATIN DESSERT POWDERS—TENTATIVE

9.7

#### PREPARATION OF SAMPLE

Sift sample thru 30-mesh sieve onto large sheet of paper, rubbing material thru sieve and tapping vigorously, if necessary. Sift sample 2 more times, mixing thoroly each time. To avoid absorption of moisture, operate as rapidly as possible, and preserve sample in air-tight container.

9.8

#### MOISTURE

Proceed as directed under 34.3, using 2 g of material prepared as directed under 9.7.

9.9

ASH.—See 34.9 or 34.10

9.10

NITROGEN.—See 9.5

9.11

#### TOTAL ACIDITY

Dissolve 1 g of material in 100 ml of recently boiled  $\text{H}_2\text{O}$  and titrate with 0.1 N NaOH, using 0.3 ml of 1% phenolphthalein soln, 2.10 (d). Report as percentage by weight of citric acid.

9.12

#### JELLY STRENGTH

To 20 g of powder add from pipet, with stirring, 100 ml of  $\text{H}_2\text{O}$  at 10–15°. Let

stand 30 min. and then bring to 45° in water bath; remove from bath and let stand 15 min. Mix by inversion, let stand 15 min., place in water bath controlled at 10° ± 0.1°, and chill 17 hours. Determine jelly strength on Bloom Gelometer with adapter on the shot cut-off mechanism (3). After adjusting shot flow to 200 g for 5 seconds (± 5 g) make determination, using the  $\frac{1}{4}$ " plummet and light weight shot receiver (paper or plastic).

#### SUCROSE (4)

##### 9.13

##### REAGENTS

- (a) *Tannin soln.*—Dissolve 5 g of tannin in 100 ml of cold H<sub>2</sub>O.  
 (b) *Lead acetate soln.*—Dissolve 100 g of Pb(C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>)<sub>2</sub> · 3H<sub>2</sub>O in 200 ml of H<sub>2</sub>O. (This makes a 30° B $\acute{e}$ . soln.)  
 (c) *Calcium carbonate.*—Free from alkali.  
 (d) *Filter Cel.*

##### 9.14

##### DETERMINATION

Place 13 g of the powder in 300 or 400 ml beaker, add 2 g of the CaCO<sub>3</sub> and 2 g of the Filter Cel, and mix well with glass rod. Add 175 ml of boiling H<sub>2</sub>O, creaming mixture with a little of the H<sub>2</sub>O at first. Stir thoroly and let stand a few min. to insure solution. Cool under cold water tap to 30°, add slowly with stirring 25 ml of the tannin soln, and let stand 5 min. (This amount of tannin soln is sufficient for most powders; if 30 ml is required, use 170 ml of H<sub>2</sub>O instead of 175 ml.) Add slowly with stirring 10 ml of the Pb acetate soln and filter on Whatman No. 2, 18.5 cm filter paper. (Total quantity of liquid used in each case is 210 ml, which yields 200 ml after evaporation and concentration. If precipitation has been conducted properly, the soln will filter readily and filtrate will be clear.) Polarize this soln in 200 mm tube at 20°.

If the powder contains a reducing sugar, delead with K<sub>2</sub>C<sub>2</sub>O<sub>4</sub>, add some Filter Cel, and filter. Invert by placing 50 ml of filtrate in 100 ml volumetric flask with 5 ml of HCl and allowing to stand overnight. After inversion, neutralize with NaOH soln, using phenolphthalein indicator. Discharge color of indicator with 0.1 N HCl. Cool to 20°, make to volume, and polarize in 200 mm tube. Use following Clerget formula modified for percentage of sucrose in gelatin dessert powders:

$$S = \frac{100[P(4) - I(8)]}{142.66 + 0.0676(m - 13) - t/2}, \text{ in which}$$

$S$  = percentage of sucrose;  $P$  = direct reading, normal soln;  $I$  = invert reading, normal soln;  $t$  = temp. at which readings are made (20°); and  $m$  = g of total solids from original sample/100 ml of invert soln (3.25 g). Simplified—

$$S = \frac{100[P(4) - I(8)]}{132}.$$

##### 9.15

##### DEXTROSE

Determine polarization due to dextrose ( $D$ ) by subtracting percentage of sucrose ( $S$ ) as found in 9.14 from direct reading of polariscope ( $P$ ) multiplied by 4:  $D = (P \times 4) - S$ .

Calculate percentage of dextrose ( $D'$ ) from following formula:

$$D' = D \times \frac{66.5}{52.5} = D \times 1.267, \text{ in which}$$

$D$  = polarization due to dextrose; 66.5 = specific rotation of sucrose; and 52.5 = specific rotation of dextrose.

## STARCH DESSERT POWDERS—TENTATIVE

9.16 PREPARATION OF SAMPLE.—*See* 9.7

## 9.17 MOISTURE

Proceed as directed under 34.2 or 34.3, using 2 g of material prepared as directed under 9.7.

9.18 ASH.—*See* 34.9 or 34.10

## 9.19 NITROGEN

Proceed as directed under 2.24, 2.25, or 2.26, using 1 g of sample. To convert to protein multiply by factor 6.25.

9.20 SUCROSE AND DEXTROSE.—*See* 9.14 and 9.15

## 9.21 STARCH

(a) *By Direct Acid Hydrolysis.*—*See* 27.33.

(b) *Polarimetric Method.*—*See* 20.47.

## SELECTED REFERENCES

- (1) U.S.D.A. Circ. 183, August 1931.
- (2) Ind. Eng. Chem., Anal. Ed. 2, 348 (1930).
- (3) Ibid., 17, 64 (1945).
- (4) Annual Report Dept. Farms and Markets, 1926, Legislative Document No. 15, p. 78 (1927).

## 10. LEATHERS—TENTATIVE

### VEGETABLE TANNED LEATHER

#### 10.1

##### PREPARATION OF SAMPLE (1)

Reduce leather sample to small pieces, mix, and then grind thru Wiley mill, using 4 mm screen. Mix thoroly and place in tightly covered container.

##### MOISTURE (2)

#### 10.2

##### Method I

Place 5–10 g of prepared sample, 10.1, in tared, wide, shallow weighing bottle (or similar dish, which can be covered tightly), and dry in electric oven 15 hours at 100–102°. Cover weighing bottle, cool in desiccator containing  $\text{H}_2\text{SO}_4$ , and weigh. The moisture in leather as received may be determined by quickly cutting a representative portion of sample into small pieces and drying as directed without further preparation.

##### Method II—By Toluene Distillation

#### 10.3

##### APPARATUS

- (a) *500 ml flask*.—Erlenmeyer, or distilling flask of Pyrex or other resistant glass.
- (b) *Receiving tube*.—Graduated in tenths of a ml.
- (c) *Liebig condenser*.—Scaled-in, straight-tube, ca 25 cm (10") long, with delivery tube ca 9.5 mm (0.375") in diam.

Assemble apparatus shown in Fig. 40, 27.4, except to use 500 ml Erlenmeyer or distilling flask instead of 250 ml flask. Clean and calibrate as directed.

#### 10.4

##### DETERMINATION

Weigh 20 g of prepared sample, 10.1, and transfer to distilling flask. Immediately add ca 200 ml of dry toluene having a b.p., under normal pressure, of 110–112°, and connect flask with receiving tube and condenser. Fill receiving tube with toluene, pouring it thru condenser. Heat distilling flask gently and distil at rate of ca 4 drops per second for exactly 2 hours. At end of 1, 1.25, 1.5, 1.75, and 2 hours' distillation, wash down condenser by pouring toluene in at top while brushing thoroly with tight-haired, close-fitting tube brush that has been boiled previously in toluene. (A long handle may be made by fastening to brush a piece of heavy Cu wire.) At end of 2 hours, disconnect receiving tube, dislodge any drops of  $\text{H}_2\text{O}$  on inside by rubbing with a piece of light Cu wire twisted at one end into a loop, and allow tube to come to room temp. Read volume of  $\text{H}_2\text{O}$  to 0.01 ml and make such calibration correction as may be necessary. Assuming that 1 ml of  $\text{H}_2\text{O}$  weighs 1 g, calculate percentage of moisture.

#### 10.5

##### TOTAL ASH (3)

Incinerate slowly 5 g of prepared sample, 10.1, at maximum of ca 600°. If difficulty is experienced in burning off C, leach residue with hot  $\text{H}_2\text{O}$ , filter on ashless filter, dry, and ignite filter and residue. Add filtrate, evaporate to dryness, and ignite. Cool in desiccator containing  $\text{H}_2\text{SO}_4$  and weigh.

The ash may be examined for acids and bases by any suitable method. Fe, Al, Mg (4), Na, Ba, Ca, and Pb are the bases, and HCl and  $\text{H}_2\text{SO}_4$  are the acids which it may be necessary to determine.



## 10.6

## INSOLUBLE ASH (5)

Quantitatively remove leather remaining after extraction of water-soluble material as directed under 10.9, dry, and ash entire charge as directed under 10.5.

## 10.7

## PETROLEUM BENZINE EXTRACT (5)

Place 5 g of prepared leather, 10.1, in fat-free paper thimble, cover with layer of fat-free cotton, and extract in Johnson or Soxhlet extractor 8–10 hours with petroleum benzine distilling at 50–80°. (Heavily greased leathers, containing 15% or more fat, will require maximum time.) Remove receiving flask, evaporate petroleum benzine on steam bath, and dry residue at 98–100° for periods of  $\frac{1}{2}$  hour each until practically constant weight is obtained. Avoid prolonged continuous heating, resulting possibly in partial volatilization or oxidation of extract.

## MINERAL ACIDITY (6)

## 10.8 (Not applicable to leathers tanned partially with minerals or syntans)

Weigh 2 g of prepared leather, 10.1, into Pt dish; add 40 ml of 0.1 *N* Na<sub>2</sub>CO<sub>3</sub> soln, mix thoroly, and evaporate to complete dryness on steam bath. Place residue in electric muffle furnace at 20–30°, slowly raise temp. of muffle to 600° ± 10° in 2 hours, and maintain at this temp. an additional hour. Remove dish, allow to cool, and carefully moisten residue with hot H<sub>2</sub>O, adding ca 25 ml, and break up lumps with glass rod. Filter into 300 ml flask thru an ashless paper and wash 4 or 5 times with hot H<sub>2</sub>O. Return filter paper and residue to its dish, dry, and incinerate in muffle furnace at 600–650° until all C is burned off. Cool, and add to residue from buret a quantity of 0.1 *N* H<sub>2</sub>SO<sub>4</sub> exactly equivalent to the Na<sub>2</sub>CO<sub>3</sub> originally added. Cover dish and place on steam bath 30 min. Transfer, or if solution is sufficiently turbid to interfere with titration, filter into flask containing the first filtrate, washing paper thoroly with hot H<sub>2</sub>O until free from acid. Cool soln and add 2 or 3 drops of methyl orange indicator (0.1 g/100 ml H<sub>2</sub>O). If soln is alkaline, no further titration is necessary, and acidity is stated as "none." If soln is acid, titrate to distinct yellow color with the 0.1 *N* Na<sub>2</sub>CO<sub>3</sub> soln. Express result as percentage of H<sub>2</sub>SO<sub>4</sub>. With each set of determinations run blank thru entire procedure, using the standard solns. If blank is over 0.3 ml, repeat determinations.

## 10.9

## EXTRACTION OF WATER-SOLUBLE MATERIAL (7)

Weigh 15 g of prepared leather, 10.1. (If fat content of sample, as determined by petroleum benzine extract, is more than 8% on dry basis, extract 15 g charge with petroleum benzine, distilling at 50–80°, and allow petroleum benzine to evaporate spontaneously from charge before proceeding with extraction of water-soluble material.) Thoroly mix with charge sufficient H<sub>2</sub>O to soak and cover leather. Transfer leather and soln to percolator that may be kept at 35°. (The percolator tubes should be 45 ± 2 mm internal diam. and 233 ± 10 mm body-height.) Extract at 35° by percolating with H<sub>2</sub>O at 35°, collecting 1 liter of percolate in 3 hours. Cool to room temp., dilute to exactly 1 liter, and mix thoroly. To prevent fermentation add a few drops of toluene to prepared extract and reserve it for determination of glucose, soluble solids, and soluble non-tannins.

## GLUCOSE (8)

## 10.10

## REAGENTS

(a) *Di-potassium phosphate*.—Use only K<sub>2</sub>HPO<sub>4</sub> that is practically free from primary and tertiary salts, has been dried in thin layers at 98–100° 16 hours, and kept

in tightly stoppered bottles. A soln of the salt should have a pH value of ca 9.0 and give a barely perceptible pink color with phenolphthalein indicator.

(b) *Neutral lead acetate soln.*—Saturated aqueous soln.

(c) *Soxhlet modification of Fehling soln.*—See 34.33.

(d) *Phenolphthalein.*—Dissolve 0.5 g of phenolphthalein in 100 ml of alcohol.

(e) *Tartaric acid.*—Grind pure tartaric acid to fine powder.

#### 10.11

##### PREPARATION OF SOLUTION

To 200 ml of prepared leather extract, 10.9, add by means of pipet 25 ml of the neutral Pb acetate soln. Shake frequently 5–10 min., filter at once thru a dry, folded filter, returning filtrate until it is clear. Keep containers and funnel covered during these operations. Add to filtrate 5.5 g of the dried  $K_2HPO_4$  (quantity of  $K_2HPO_4$  must be 4.5–6.5 g). Shake frequently 3–5 min., until all phosphate has dissolved. Filter thru dry, folded filter, returning first runnings until filtrate clears, and letting funnel drain well. Pipet 150 ml of the filtrate into 500 ml Erlenmeyer flask, and add by means of pipet 7.5 ml of HCl. Also add ca 25 mg of powdered stearic acid or 5–10 drops of kerosene to control frothing, and boil under reflux condenser exactly 2 hours. (If foaming occurs, turn off flame, and when foaming subsides relight immediately. No further trouble should be experienced. After hydrolysis the acid soln may stand at laboratory temp. overnight without risk of loss of sugar.) Cool to 10–15°, add 2 drops of the phenolphthalein indicator, carefully neutralize with NaOH (1+1) added from buret, and add 0.5 ml in excess. *Without delay* transfer soln to 200 ml volumetric flask, complete to volume with  $H_2O$ , and filter thru double filter, returning filtrate until it is clear. *During filtration keep filtrate just acid by addition from time to time of small quantities of the pulverized pure tartaric acid. Immediately determine dextrose in soln.*

#### 10.12

##### DETERMINATION

Pipet 50 ml of the prepared soln into mixture of 25 ml of the Cu soln and 25 ml of the alkaline tartrate soln and proceed as directed under 34.39. Express results as percentage of glucose on leather basis, the 50 ml aliquot being equivalent to 0.5 g of leather.

#### 10.13

##### SOLUBLE SOLIDS

If  $H_2O$  extract, prepared as directed under 10.9, is clear, proceed as directed under 11.2; if it is cloudy, proceed as directed under 11.5.

#### 10.14

##### SOLUBLE NONTANNINS

Proceed as directed under 11.8, except to use 25 g of prepared wet hide powder for 200 ml of leather extract. Express results as percentage of original leather.

#### 10.15

##### SOLUBLE TANNIN

The percentage of soluble tannin is difference between 10.13 and 10.14.

#### 10.16

##### NITROGEN.—See 2.24

#### 10.17

##### HIDE SUBSTANCE

Multiply percentage of N, 10.16, by factor 5.62, to convert to percentage of hide substance.

## 10.18

## COMBINED TANNIN

Deduct sum of percentages of moisture, 10.2 or 10.4; insoluble ash, 10.6; petroleum benzine extract, 10.7; soluble solids, 10.13; and hide substance, 10.17, from 100. The remainder is percentage of combined tannin.

## SELECTED REFERENCES

- (1) J. Am. Leather Chem. Assoc., 13, 232 (1918); 14, 321 (1919); 18, 154 (1923); 23, 412 (1928).
- (2) Ibid., 16, 547 (1921); 17, 262 (1922); 19, 568 (1924); 20, 334 (1925); 21, 435 (1926); 22, 265 (1927); J. Assoc. Official Agr. Chem., 10, 31, 143 (1927).
- (3) J. Am. Leather Chem. Assoc., 13, 7 (1918); 14, 243 (1919); 15, 130, 270 (1920).
- (4) Ibid., 16, 595 (1921); 17, 274, 592 (1922).
- (5) Ibid., 14, 140, 499, 507 (1919); 16, 458 (1921); 17, 292, 540 (1922); J. Soc. Leather Trades Chem., 4, 300 (1920).
- (6) J. Am. Leather Chem. Assoc., 14, 330 (1919); 18, 430 (1923); 28, 580 (1933); 29, 259 (1934).
- (7) Ibid., 14, 133, 488 (1919); 15, 581 (1920); 16, 264, 491 (1921); 17, 220 (1922); 34, 369 (1939).
- (8) Ibid., 7, 645 (1912); 9, 421 (1914); 15, 411 (1920); 16, 480 (1921); 17, 284 (1922); 18, 262, 459 (1923); 19, 237, 339 (1924).

## 11. TANNING MATERIALS (1)—TENTATIVE

### EXTRACTS

#### 11.1

##### PREPARATION OF SOLUTION

(a) *Solid and powdered extracts*.—Grind sample, if necessary, as rapidly as possible in porcelain mortar until all will pass 10-mesh sieve of Cu or brass; mix thoroly and bottle. Weigh rapidly, in suitable glass container, quantity of sample containing ca 4 g of tannin (not less than 3.75, not more than 4.25 g). Pour ca 200 ml of  $H_2O$  at 95° into 1 liter flask, then place container with extract in funnel resting in neck of flask. Add sufficient  $H_2O$  at 95° to the extract to fill container. Stir well, allow solid particles to settle, and decant liquid into flask. Repeat addition of  $H_2O$  at 95°, stirring and decanting until all of extract has been dissolved and washed into flask. Add  $H_2O$  until final volume is ca 900 ml. (The dissolving and decanting should be done as rapidly as possible. During dissolving operation soln in flask must not cool below 80°; apply heat if necessary to maintain this temp.) Stir well, allow to stand overnight at temp. not below 23°, bring to 23° by placing flask in  $H_2O$  (temp. not less than 22°), and dilute to 1 liter.

(b) *Liquid extracts*.—Let sample come to 20–30°, mix thoroly, and weigh rapidly charge yielding same quantity of tannin as is specified under (a). Dissolve by washing into liter flask (containing ca 200 ml of  $H_2O$  at 95°) with ca 700 ml of  $H_2O$  at 95°. Allow to cool and dilute to 1 liter at 23°, as directed under (a).

After preparation of soln proceed at once with analysis.

#### 11.2

##### TOTAL SOLIDS

Thoroly mix prepared soln, 11.1, and pipet at once 100 ml into weighed flat-bottomed glass dish, 2½–3" in diam. (2). Evaporate and dry 16 hours in combined evaporator and dryer (3) at 98–100°, or evaporate on steam bath and dry 12 hours on bottom of water oven at 98–100°. Remove immediately to desiccator containing  $H_2SO_4$  (not more than 2 dishes in one desiccator) and weigh rapidly when cooled. Calculate percentage of total solids.

##### SOLUBLE SOLIDS

#### 11.3

##### REAGENT

*Kaolin*.—Should be neutral to phenolphthalein and should not yield more than 1 mg of soluble solids/100 ml of filtrate of 1% suspension in  $H_2O$  after an hour's digestion at 23°.

#### 11.4

##### PREPARATION OF FILTER

To ca 75 ml of prepared soln, 11.1, add 1 g of the kaolin. Stir, and pour immediately into single, 15 cm No. 1F Swedish filter (4). (These papers must be pleated by hand as they are not available in folded form.) Return filtrate to paper when ca 25 ml has run thru and repeat operation for 1 hour, thus transferring all kaolin to paper. At end of an hour discard soln on filter by siphoning it off, disturbing the kaolin as little as possible. An ordinary wash bottle serves well for this purpose.

#### 11.5

##### DETERMINATION

Bring ca 150 ml of original prepared soln, 11.1, to exactly 23°. Fill filter, prepared as directed under 11.4, with this soln and discard filtrate until it runs thru clear. Keep filter full, temp. of filtering soln at 23–25°, and the funnel and receiving vessel

covered. Pipet at once 100 ml of the clear filtrate into weighed dish, evaporate, and dry as directed under 11.2. Calculate percentage of soluble solids.

## 11.6

## INSOLUBLE SOLIDS

Percentage of insoluble solids = total solids, 11.2,—soluble solids, 11.5.

## NONTANNINS

## 11.7

## REAGENT

*Hide powder (5).*—Of wooly texture, well delimited; ash content less than 0.3%. Filtrate from 7.0 g of air-dry powder, suspended in 100 ml of 0.1 *N* KCl and allowed to stand 24 hours, shall have a pH of not less than 5.0 nor more than 5.4.

Calculate quantity of air-dried hide powder that will be required for number of determinations to be made, on basis of 12.5 g of H<sub>2</sub>O-free powder for each determination. Increase this calculated amount by 10 g of air-dried hide powder to provide a sufficient quantity for determination of moisture in wet chromed hide powder and also for a working leeway.

Digest total quantity of air-dried hide powder with 10 times its weight of H<sub>2</sub>O until thoroly soaked. For each g of air-dried hide powder, so digested, add 1 ml of 3% chrome alum soln, K<sub>2</sub>SO<sub>4</sub>Cr<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>·24H<sub>2</sub>O, and either agitate frequently several hours and let stand overnight, or agitate in some form of mechanical shaker for 1 hour. Transfer to strong linen filter and squeeze thoroly; using linen filter as a bag, leave hide powder in it and digest 15 min. with quantity of H<sub>2</sub>O equal to 15 times weight of air-dried hide powder used. Filter, and squeeze to ca 73% of H<sub>2</sub>O, using press if necessary. (Strong pressure is required to reduce H<sub>2</sub>O content below 70%.) Repeat digestion and filtration 3 times. The wet chromed hide powder, as finally prepared, should contain as nearly as possible 73% of H<sub>2</sub>O. Moisture content must be not less than 72% nor more than 74%. Determine moisture in 20 g of squeezed hide powder as directed under 11.2.

## 11.8

## DETERMINATION

Place 46 g of prepared wet hide powder, 11.7, in shaker bottle of suitable capacity; add 200 ml of prepared tanning soln, 11.1, and shake immediately 10 min. in mechanical shaker. Squeeze at once thru linen; add 2 g of kaolin, 11.3, to filtrate that contains the nontannins; stir; and filter thru a single, *folded* 18.5 cm filter paper (No. 1F Swedish preferred), refiltering until filtrate is clear. Test filtrate with gelatin-salt soln (1% gelatin and 10% salt), and if precipitate forms, report fact. Pipet 100 ml of filtrate into weighed dish and evaporate as directed under 11.2. Correct weight of nontannin residue for dilution caused by H<sub>2</sub>O retained in wet hide powder. Calculate percentage of nontannins.

## 11.9

## TANNIN

Percentage of tannin = soluble solids, 11.5,—nontannins, 11.8.

## SUGARS (c)

## 11.10

## PREPARATION OF SOLUTIONS

To 400 ml of prepared soln, 11.1, add 50 ml of Pb acetate soln, 10.10(b), shake well, and let stand 5–10 min. Filter thru folded filter (18.5 cm), returning filtrate until it is clear. Let the filter drain ca 30 min. after all soln has been poured. Remove excess Pb from filtrate with dried K<sub>2</sub>HPO<sub>4</sub>, 10.10(a), using K<sub>2</sub>HPO<sub>4</sub> in proportion of 5 g to 200 ml of filtrate. (Measure filtrate in graduated cylinder; usually 360–380

ml will be obtained, requiring 9–9.5 g of  $K_2HPO_4$ . Weigh  $K_2HPO_4$  to within 0.1 g.) After adding  $K_2HPO_4$ , shake well 4 or 5 min. and filter thru folded filter (18.5 cm).

## 11.11

## DETERMINATION

(a) *Reducing sugars*.—Place in flask 100 ml of the clarified delead ed soln, add 33.3 ml of  $H_2O$ , and if reduction is not made at once, 8–10 drops of toluene; shake well and stopper with plug of cotton. Keep in cool place and make reduction within 24 hours. When ready for reduction, filter if toluene has been added. Determine reducing sugars in duplicate 50 ml aliquots, as directed under 34.39. After correcting weight of  $Cu_2O$  for blank of the Fehling soln, find equivalent mg of dextrose from 44.11. To express as percentage of dextrose, multiply mg of dextrose by 3 and divide result by g of sample/liter of prepared soln, 11.1.

(b) *Total sugars*.—Place in 500 ml Erlenmeyer flask 150 ml of the clarified, delead ed soln, add 7.5 ml of HCl, and boil under reflux condenser exactly 1 hour. (If foaming occurs, add 5–10 drops of kerosene.) After boiling an hour, remove flask, stopper loosely when moderately cool, and let stand until ready for reduction, usually overnight. Cool soln in ice water 20–30 min., add 2 drops of phenolphthalein soln, 10.10(d), carefully neutralize with NaOH (1+1), and add HCl dropwise until color of indicator is just discharged. After bringing soln to room temp., transfer to 200 ml flask, make to mark, mix, and filter until clear. Reduce the Fehling soln with duplicate 50 ml aliquots and calculate results as directed under (a).

(c) *Non-reducing sugars*.—Percentage of non-reducing sugars is difference between total sugars (b), and reducing sugars (a).

## DETECTION OF SULFITE-CELLULOSE (7)

## 11.12

## REAGENT

*Sulfite-cellulose soln*.—Dissolve 0.5 g of the total solids derived from sulfite-cellulose in 1 liter of  $H_2O$  and add sufficient tanning material, free from sulfite-cellulose, to give concentration of 3.75–4.25 g of tannin/liter.

## 11.13

## DETERMINATION

Place 5 ml of prepared tanning soln, 11.1, in test tube. Add 0.5 ml of aniline and shake well; add 2 ml of HCl and mix again. Compare precipitate formed with that produced when sulfite-cellulose soln is similarly treated. In predetermined absence of the synthetic tanning materials known as syntans, sulfite-cellulose is considered to be present if volume of precipitate approximately equals or exceeds that of comparison soln.

## LIQUORS

## 11.14

## PREPARATION OF SOLUTION

Dilute the liquor with  $H_2O$  at 20–30° to contain ca 0.7 g of solids in 100 ml of soln. If liquor does not give proper soln with  $H_2O$  at 20–30°, dilute with  $H_2O$  at 80° and cool to 20°, as directed under 11.1(a).

## 11.15

## TOTAL SOLIDS.—See 11.2

## 11.16

## SOLUBLE SOLIDS.—See 11.5

## 11.17

## NONTANNINS

Proceed as directed under 11.8, using quantity of wet chromed hide powder that will give ratio between tannin and hide powder shown in following table (8):

TANNIN RANGE PER 100 ML	DRY HIDE POWDER PER 200 ML
<i>gram</i>	<i>gram</i>
0.35–0.45	9.0–11.0
0.25–0.35	6.5– 9.0
0.15–0.25	4.0– 6.5
0.00–0.15	0.0– 4.0

## TOTAL ACIDITY

## 11.18

## REAGENTS

(a) *Hematin indicator*.—Digest 0.5 g of hematin in 100 ml of cold neutral alcohol.

(b) *Gelatin soln*.—Soak 10 g of gelatin in  $H_2O$  at room temp. 1–2 hours and warm slightly, not exceeding  $50^\circ$ , to complete soln; add 25 ml of alcohol, and dilute. If gelatin soln is acid or alkaline, neutralize with 0.1 *N* NaOH or 0.1 *N* acetic acid, respectively, using hematin indicator, and dilute to 1 liter.

(c) *Kaolin*.—Digest with HCl (1+9), wash until it complies with tests given under 11.3, dry, and preserve in a tightly stoppered bottle.

## 11.19

## DETERMINATION (9)

Add 50 ml of the gelatin soln to 25 ml of the tanning liquor in a stoppered cylinder, dilute with  $H_2O$  to 250 ml, add 15 g of the kaolin, and shake vigorously. Allow to settle at least 15 min., remove 30 ml of supernatant liquid, dilute with 50 ml of  $H_2O$ , and titrate with 0.1 *N* NaOH, using the hematin indicator. 1 ml of 0.1 *N* NaOH = 0.2% of acid, calculated as acetic, in liquor.

## RAW AND SPENT MATERIALS

(Under raw materials are included woods, barks, leaves, etc.)

## 11.20

## MOISTURE IN SAMPLE AS RECEIVED

Cut or break up large pieces and mix sample rapidly to avoid change in moisture content. Dry as directed under 11.2, a suitable weighed quantity, dependent upon physical condition and moisture content of sample.

## 11.21

## PREPARATION OF SAMPLE

Dry remainder of sample at temp. not above  $60^\circ$ , and grind to pass thru 20-mesh sieve.

## 11.22

## MOISTURE IN PREPARED SAMPLE

Dry 10 g of prepared sample, 11.21, as directed under 11.2, and calculate all results to "as-received," "air-dried," or "moisture-free" basis, as desired.

## 11.23

## EXTRACTION

(a) *Woods, barks, and spent materials*.—Weigh quantity of sample that will give an extract containing as nearly as possible 4 g of tannin per liter. Transfer to beaker and wet thoroly with hot  $H_2O$ . Place perforated porcelain plate in tin-lined Cu extractor of general form shown in Fig. 14; on plate place layer of cotton and wet thoroly with  $H_2O$ . Connect extractor with 1000 ml Erlenmeyer flask (G) containing 800 ml of  $H_2O$ , close stopcock E, connect D by delivery tube to 1000 ml

graduated flask and close D. Wash material into extractor with minimum amount of hot  $H_2O$ . Open D and return percolate thru extractor 2 or 3 times, or until it is practically clear. Place layer of cotton on top of the material. Connect metal cap B and condenser A so that condensate will drop onto layer of cotton. Boil  $H_2O$  in G and collect 500 ml of percolate in the graduated flask in 2 hours. Close D, open E, and continue boiling 14 hours, applying heat so that ca 330 ml of  $H_2O$  will be condensed per hour. Transfer extract in G to graduated 1 liter flask, heat to  $80^\circ$ , cool as directed under 11.1(a), and dilute to mark.

(b) *Materials other than woods, barks, and spent materials.*—Weigh quantity of sample sufficient to give 2 liters of extract containing 4 g of tannin per liter. Place in extractor described under (a), digest 1 hour with  $H_2O$  at room temp., and start the extraction. Keep stopcock E closed and collect entirely thru the side tube D 2 liters of percolate in ca 7 hours. Heat percolate to  $80^\circ$ , cool as directed under 11.1(a), and dilute to mark.

#### 11.24 ANALYSIS OF EXTRACT

Proceed as directed under 11.2–11.9, inclusive. With solns more dilute than specified (often the case with spent materials) reduce quantity of hide powder used in determination of nontannins in accordance with concentration of soln and schedule given under 11.17.

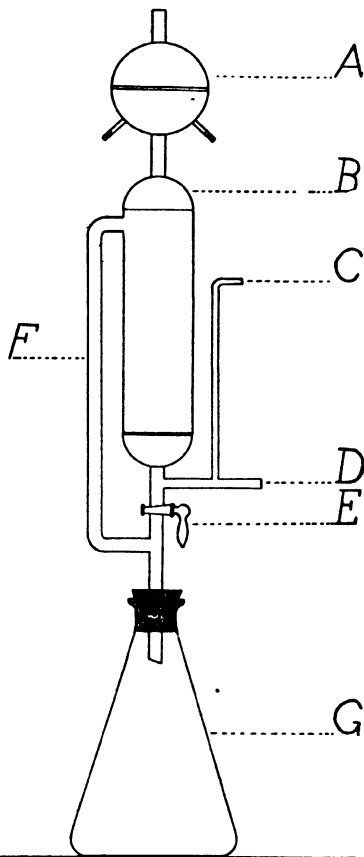


FIG. 14.—APPARATUS FOR EXTRACTING TANNING MATERIALS

#### SELECTED REFERENCES

- (1) The proceedings of the A.O.A.C. that deal with the early development of the methods of analysis of tanning materials will be found in Bureau of Chemistry Bulletins Nos. 43, 47, 49, 51, 56, 62, 67, 73, 81, and 90. These have been assembled in J. Am. Leather Chem. Assoc., 15, 1a–127 (1920).
- (2) J. Am. Leather Chem. Assoc., 31, 300 (1936).
- (3) Ibid., 1, 32 (1906); 9, 442 (1914).
- (4) Ibid., 10, 282 (1915).
- (5) The official hide powder of the American Leather Chemists Association is prepared only by the Hide Powder Division of the Keystone Tanning and Glue Co., Ridgeway, Pa.
- (6) J. Am. Leather Chem. Assoc., 23, 91 (1928).
- (7) Ibid., 9, 36, 130 (1914).
- (8) Ibid., 5, 5 (1910).
- (9) Ibid., 3, 85 (1908); 4, 194 (1909).



## 12. PLANTS

### 12.1

#### DIRECTIONS FOR SAMPLING (1)—OFFICIAL

When more than one plant is sampled, include in sample a sufficient number of plants to insure that it adequately represents average composition of entire lot of plants sampled. This number cannot be stated definitely; it will depend upon the variability in composition of the plants. Details of procedure must be determined by purpose for which sample is taken.

### 12.2

#### PREPARATION OF SAMPLE (1)—OFFICIAL

(a) *For mineral constituents.*—Thoroughly remove all foreign matter from material, especially adhering soil or sand, but to prevent leaching avoid excessive washing. Air or oven dry as rapidly as possible to prevent decomposition or loss in weight by respiration, grind, and preserve in tightly stoppered bottles. If results are to be expressed on fresh weight basis, record weights of sample before and after drying. When determinations of Cu, Mn, Zn, Fe, Al, etc. are to be made, take precautions to prevent contamination of sample by dust during drying and from grinding and sieving machinery.

(b) *For carbohydrates.*—Thoroughly remove all foreign matter and rapidly grind or chop material into fine pieces. Add weighed sample to sufficient hot redistilled alcohol to which sufficient precipitated  $\text{CaCO}_3$  has been added to neutralize acidity, using sufficient alcohol so that final concentration, allowing for  $\text{H}_2\text{O}$  content of sample, will be ca 80%. Heat close to b.p. on steam or water bath 30 min., stirring frequently. Samples may be stored until needed for analysis.

### 12.3

#### MOISTURE—TENTATIVE.—See 27.3, 27.7, or 27.8

### 12.4

#### ASH—TENTATIVE.—See 34.9 or 34.10; 27.9

### 12.5

#### SAND AND SILICA—OFFICIAL

Ignite 10–50 g of substance in flat-bottomed Pt dish in muffle, at temp. not exceeding dull redness, until residue is white or nearly so. (Pt dishes must be used with caution in ashing plant materials high in Fe; for such materials use well-glazed porcelain crucibles and run a blank determination.) Moisten with 5–10 ml of HCl, boil ca 2 min., evaporate to dryness, and heat on steam bath 3 hours to render the  $\text{SiO}_2$  insoluble. Moisten residue with 5 ml of HCl, boil 2 min., add ca 50 ml of  $\text{H}_2\text{O}$ , heat on water bath a few minutes, filter thru hardened filter, and wash thoroughly. To this filtrate add filtrate and washings from alkali-soluble  $\text{SiO}_2$  determination (b) and dilute to 200 ml. Designate as soln A.

(a) *Sand.*—Wash the residue from filter into Pt dish and boil ca 5 min. with ca 20 ml of a saturated  $\text{Na}_2\text{CO}_3$  soln; add a few drops of 10% NaOH soln; allow mixture to settle; and decant thru ignited and weighed Gooch crucible. Boil residue in dish with another 20 ml portion of the  $\text{Na}_2\text{CO}_3$  soln and decant as before. Repeat process. Transfer residue to Gooch crucible and wash thoroughly, first with hot  $\text{H}_2\text{O}$ , then with a little HCl (1+4), and finally with hot  $\text{H}_2\text{O}$  until free from Cl. Dry filter and contents, ignite, and weigh as sand. Confirm by microscopic examination.

(b) *Alkali-soluble  $\text{SiO}_2$ .*—Combine alkaline filtrate and washings, acidify with HCl, evaporate to dryness, add 5 ml of HCl, again evaporate, and dehydrate by heating to 110–120° 2 hours. Moisten residue with 5–10 ml of HCl, boil ca 2 min., add ca 50 ml of  $\text{H}_2\text{O}$ , heat on water bath 10–15 min., filter thru ashless filter or ignited and weighed Gooch crucible, wash with hot  $\text{H}_2\text{O}$ , ignite, and weigh as  $\text{SiO}_2$ . Add filtrate to soln A.

## 12.6

## IRON AND ALUMINUM (s)—OFFICIAL

Take aliquot of soln A, 12.5, containing ca 40 mg of Fe- and  $\text{AlPO}_4$ . Oxidize Fe by use of  $\text{HNO}_3$ , Br water,  $\text{H}_2\text{O}_2$ , or other standard procedures. If soln does not already contain an excess of phosphate, add to aliquot containing ca 40 mg of Fe- and  $\text{AlPO}_4$ , 0.5 g of  $(\text{NH}_4)_2\text{HPO}_4$ , stir until dissolved, and make up to 50 ml with  $\text{H}_2\text{O}$ . Add a few drops of thymol blue and then add  $\text{NH}_4\text{OH}$  until soln just turns yellow. Run in 0.5 ml of  $\text{HCl}$ , follow with 25 ml of 25%  $\text{NH}_4$  acetate soln, and stir. Let stand at room temp. until precipitate settles (ca 1 hour). Filter, and wash 10 times with hot 5%  $\text{NH}_4\text{NO}_3$  soln. Ignite, and weigh as Fe- and  $\text{AlPO}_4$ .

Fuse ignited precipitate in Pt crucible with ca 4 g of a mixture of equal parts of  $\text{Na}_2\text{CO}_3$  and  $\text{K}_2\text{CO}_3$ . When fusion is complete, allow crucible to cool, add 5 ml of  $\text{H}_2\text{SO}_4$ , and heat until copious fumes of  $\text{SO}_3$  are given off. Cool, transfer to flask, add  $\text{H}_2\text{O}$ , and digest until soln is clear. Reduce Fe with Zn, cool, and titrate with 0.1 N  $\text{KMnO}_4$  soln. Correct for blank and report as percentage of  $\text{Fe}_2\text{O}_3$ . Calculate to phosphate and subtract from total Fe- and  $\text{AlPO}_4$  to obtain  $\text{AlPO}_4$ . Correct for blank and report as  $\text{Al}_2\text{O}_3$ .

## METHODS FOR IRON ONLY

## Colorimetric Method(s)—Tentative

## 12.7

## REAGENTS

- (a) *Acetic acid*.—2 M. Dilute 120 g of acetic acid to 1 liter with  $\text{H}_2\text{O}$ .
- (b) *Ammonium citrate soln*.—1%. Dissolve 1 g of  $\text{NH}_4$  citrate in  $\text{H}_2\text{O}$  and dilute to 100 ml.
- (c) *Bromophenol blue indicator*.—0.04%. Grind 0.1 g of bromophenol blue in mortar with 3 ml of 0.05 N  $\text{NaOH}$  soln, transfer to volumetric flask, and dilute to 250 ml with  $\text{H}_2\text{O}$ .
- (d) *Buffer solns*:
  - (1) *Soln of pH 3.5*.—Mix 6.4 ml of 2 M Na acetate soln with 93.6 ml of 2 M acetic acid and dilute to 1 liter.
  - (2) *Soln of pH 4.5*.—Mix 43 ml of 2 M Na acetate soln with 57 ml of 2 M acetic acid and dilute to 1 liter.
- (e) *Hydroquinone soln*.—Dissolve 1 g of hydroquinone in 100 ml of buffer soln, pH 4.5. Keep in refrigerator or other cool place, and discard as soon as any color develops.
- (f) *O-phenanthroline soln*.—Dissolve 1 g of o-phenanthroline monohydrate in  $\text{H}_2\text{O}$ , warming if necessary to effect soln, and dilute to 400 ml.
- (g) *Sodium acetate soln*.—2 M. Dissolve 272 g of  $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.
- (h) *Standard iron soln*.—1 mg of Fe/ml. Dissolve 1 g of electrolytic Fe in 50 ml of 10%  $\text{H}_2\text{SO}_4$ , warming if necessary to hasten reaction. Cool, and dilute to 1 liter with  $\text{H}_2\text{O}$ .

## 12.8

## DETERMINATION

Pipet similar aliquots of soln A, 12.5, into 25 ml volumetric flask and a test tube or small Erlenmeyer flask. Add 5 drops of the bromophenol blue indicator to aliquot in test tube and titrate with the 2 M Na acetate soln until color matches that of equal volume of buffer soln of pH 3.5 containing same quantity of indicator. Add 1 ml of the hydroquinone soln and 2 ml of the o-phenanthroline reagent to aliquot in volumetric flask, and adjust pH of the contents to 3.5 by adding the same volume of Na acetate soln as was found necessary for aliquot in test tube. If turbidity de-

velops upon adjustment of pH of aliquot in test tube, add 1 ml of the ammonium citrate soln to volumetric flask before adding the Na acetate soln. Make to volume, mix, and let stand for 1 hour to assure complete color development. Take photoelectric colorimeter reading or compare with suitable standard in visual colorimeter to determine concentration of Fe.

If soln A is not available, weigh samples of finely ground plant material (1–5 g, depending on the Fe content) into porcelain crucibles with smooth inner surfaces, and ash overnight at 500°–550° in electric muffle furnace. Cool, add 5 ml of HCl (1+1), and heat on steam bath for 15 min. to effect soln of Fe and to hydrolyze pyrophosphate. Filter into 100 ml volumetric flask. Transfer insoluble residue to the filter and wash 5 times with 3 ml portions of hot HCl (1+100), then with hot H<sub>2</sub>O until washings are free of Cl. Ignite paper and any remaining C in Fe-free Pt crucible. After cooling add 2 drops of H<sub>2</sub>SO<sub>4</sub> and 1 ml of HF, and carefully evaporate to SO<sub>2</sub> fumes. Cool, add a few drops of HCl (1+1), and warm. Filter and wash as before into same volumetric flask, make to volume, mix, and continue as directed above.

## 12.9

### SUPPLEMENTARY INFORMATION

Aliquot selected should contain an amount of Fe suitable for range of colorimeter to be used. For a photoelectric colorimeter this quantity will depend on the light filter and the thickness of the absorption cells employed. A No. 430 dark-shade, blue-green, Corning glass light filter, ca 12.5 mm thick (obtained by using 2 moulded filters of one-half this thickness) is satisfactory for this determination, and when used with 1 cm absorption cells in a Cenco-Sheard-Sanford photometer, reliable range will be from .02 to .1 mg of Fe in 25 ml of soln. For a photometric instrument it is necessary to prepare a curve relating per cent of light transmitted and mg of Fe in 25 ml. This is done by treating a series of solns containing varying quantities of Fe, which cover the usable range of the instrument, in exactly same manner as described for the unknowns, determining their respective transmission readings, and plotting these against the corresponding concentrations of Fe. Water may be used as the comparison liquid, and blanks run to correct for amount of Fe in reagents used, or the blank soln itself may be made the basis of comparison. For a visual colorimeter a range of 0.2–0.5 mg of Fe in 25 ml is suggested. Series of standards covering this range should be prepared simultaneously with the unknowns, and each unknown compared with a standard that does not vary more than 25% from it in concentration.

## 12.10

### *Titrimetric Method 3(a)—Official, first action*

Take appropriate aliquot of soln A or of soln prepared as directed under 12.8, and oxidize the Fe by adding dropwise a soln of KMnO<sub>4</sub> (1+1,000) until a very, very faint permanganate color persists. Add 5 ml of 10% NH<sub>4</sub>CNS soln and titrate with dilute TiCl<sub>3</sub> soln to disappearance of red color. (Appropriate TiCl<sub>3</sub> soln can be prepared by boiling 5–10 ml of 20% TiCl<sub>3</sub> soln with 50 ml of HCl for a few minutes, cooling, and diluting to 1 liter. This soln should be standardized with a known Fe soln, kept in dark in well-filled container, and standardized against the Fe soln each time it is used, or every few hours when large number of determinations are being made. Discard when decomposition becomes evident.)

## 12.11

### MICRO METHOD FOR ALUMINUM ONLY (4)—TENTATIVE

Take an aliquot of soln A, 12.5, containing ca 0.05 mg of Al. Oxidize the Fe by boiling with a few drops of HNO<sub>3</sub> and transfer to conical centrifuge tube of ca 25 ml

capacity with marks at 15, 20, and 25 ml. If quantity of Fe is very small, add  $\text{Fe}_2\text{Cl}_6$  soln equivalent to ca 1 mg of iron; or if quantity of phosphate present is small, add ca 0.1 g of  $(\text{NH}_4)_2\text{HPO}_4$  to insure complete precipitation of the Fe and Al. Dilute contents to ca 15 ml with  $\text{H}_2\text{O}$ ; neutralize with  $\text{NH}_4\text{OH}$ , using a drop of dilute methyl red as indicator soln; and add 1 ml of a saturated  $\text{NH}_4$  acetate soln. Place tube in water bath until precipitate begins to settle (ca 10 min.), centrifuge, decant, and discard supernatant liquid. Dissolve precipitate in 1 ml of ca 6 N HCl with warming when necessary and dilute to 15 ml. Cool, add 1.25 ml of acetic acid and 5 ml of 6 N NaOH (special Al-free), wash down sides, and fill to 25 ml mark. Let stand ca 1 hour and centrifuge. The precipitate contains the Fe and the soln the Al.

Transfer to 50 ml volumetric flask as large an aliquot as can be drawn off. Add  $\text{H}_2\text{O}$  to make ca 20 ml, a small piece of litmus paper, and finally HCl (1+9) until litmus paper just turns red. Determine Al as follows: add 5 ml of 5 N  $\text{NH}_4$  acetate, 5 ml of 1.5 N HCl, and 2 ml of 0.1% soln of the dye Aluminon (ammonium salt of aurintricarboxylic acid) and place in water bath at ca 80° for 10 min. Add 5 ml of 5 N  $\text{NH}_4\text{Cl}$ , cool to room temp., add 5 ml of 3.2 N  $(\text{NH}_4)_2\text{CO}_3$  while shaking gently, fill to mark with  $\text{H}_2\text{O}$ , and mix. (At this point reaction should be 7.1–7.3 and red color of blank should disappear in ca 15 min. The exact concentration of reagents is not important, but final pH is, and amount of  $(\text{NH}_4)_2\text{CO}_3$  necessary to bring soln to above pH should be determined by neutralizing similar solns without adding the dye.) Simultaneously with above procedure run a standard (or standards if necessary) containing given quantity of Al. After allowing mixture to stand 20 min. for excess dye to decolorize, compare color intensities and read amount of Al from a curve plotted as described in following paragraph.

If only a small number of determinations are to be made, prepare 4 standards containing 0.01, 0.03, 0.05, and 0.07 mg of Al, respectively, and run these with samples. Compare all these solns with standard containing 0.03 mg of Al and calculate results to colorimeter reading of 30 for this standard. Arbitrarily give 0.005 mg of Al a reading of 100 and with this and the 4 readings on standards plot a curve. Read quantity of Al in each sample from this curve. If the determinations are to extend over a period of time, it is advisable to make them on several series of standards and plot a curve from the average of these results. It is then necessary to run only 1 standard each time determinations are to be made, and results can be read from curve.

#### CALCIUM—OFFICIAL

##### 12.12

##### *Macro Method (5)*

Transfer aliquot of soln A, 12.5, to 200 ml beaker, add  $\text{H}_2\text{O}$  if necessary to make to 50 ml, heat to boiling, and add 10 ml of saturated  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  soln and a drop of methyl red soln. Almost neutralize with  $\text{NH}_4\text{OH}$  and boil until precipitate is coarsely granular. Cool, add  $\text{NH}_4\text{OH}$  (1+4) until color is faint pink (pH 5.0) and allow to stand at least 4 hours. Filter, and wash with  $\text{H}_2\text{O}$  at room temp., until filtrate is free from oxalates. Break point of filter with a Pt wire and wash precipitate into beaker in which the Ca was precipitated with hot  $\text{H}_2\text{SO}_4$  (1+4) and hot  $\text{H}_2\text{O}$ . Add ca 10 ml of  $\text{H}_2\text{SO}_4$  (1+4), heat to ca 90°, add ca 50 ml of hot  $\text{H}_2\text{O}$ , and titrate with 0.05 N  $\text{KMnO}_4$ . Finally add filter paper to soln and complete titration.

##### 12.13

##### *Micro Method (6)*

Ignite 2 g of substance in small crucible in muffle at dull red heat. Dissolve ash in HCl (1+4) and transfer to 100 ml beaker. Add 5 ml of HCl and evaporate to dryness on steam bath to dehydrate the  $\text{SiO}_2$ . Moisten residue with 5 ml of HCl,

add ca 50 ml of  $\text{H}_2\text{O}$ , heat a few minutes on steam bath, transfer to 100 ml volumetric flask, cool quickly to room temp., make to volume, shake, and filter, discarding first portion of filtrate. Pipet 15 ml aliquot into conical-tipped centrifuge tube containing 2 ml of saturated  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  soln and 2 drops of 0.05% methyl red. Add 2 ml of acetic acid (1+4), rotating tube to mix contents thoroly. Add, while intermittently rotating tube,  $\text{NH}_4\text{OH}$  (1+4) until soln is faintly alkaline, after which add a few drops of the acetic acid with dropper until color is adjusted to faint pink (pH 5.0). (It is important at this point to rotate tube so that last bit of liquid in conical tip is color required.) Allow mixture to stand at least 4 hours and whirl tube in centrifuge 15 min. (Precipitate should then be in a firm lump in tip of tube.) Remove supernatant liquid by means of the suction device (Fig. 15), taking care not to disturb precipitate. Wash precipitate by adding 2 ml of  $\text{NH}_4\text{OH}$  (1+49), rotating tube to break up precipitate. (It may be necessary to jar tube sharply.) Return tube to centrifuge 10 min., and again remove supernatant liquid and wash with reagent as before. Repeat this operation until precipitate has been washed 3 times. When supernatant liquid has been removed after final centrifuging add 2 ml of  $\text{H}_2\text{SO}_4$  (1+4) to tube, break up precipitate as before, heat on steam bath to  $80-90^\circ$ , and titrate in tube with 0.02  $N$   $\text{KMnO}_4$ , rotating liquid during titration to attain a proper end point. If tube cools below  $60^\circ$  during addition of the  $\text{KMnO}_4$ , reheat it in steam bath a few minutes and complete titration. Run blank on identical amount of  $\text{H}_2\text{SO}_4$  in a similar tube heated to same temp. to determine quantity of 0.02  $N$   $\text{KMnO}_4$  necessary to give color of end point. Subtract this value from buret reading. 1 ml of 0.02  $N$   $\text{KMnO}_4 = 0.000400$  g of Ca. Report as percentage of Ca.

#### 12.14 MAGNESIUM (7)—OFFICIAL

To combined filtrate and washings from Ca determination, 12.12, add 30 ml of  $\text{HNO}_3$  and evaporate to dryness to decompose the  $\text{NH}_4$  salts. Take up with 5 ml of  $\text{HCl}$  and make to ca 100 ml with  $\text{H}_2\text{O}$ . Add 5 ml of 10% Na citrate soln and 10 ml of 10%  $(\text{NH}_4)_2\text{HPO}_4$  soln, or enough to precipitate all the Mg. Add  $\text{NH}_4\text{OH}$  (1+4) with constant stirring (using policeman) until soln is faintly alkaline and precipitate forms; then add 25 ml of  $\text{NH}_4\text{OH}$ , stir vigorously until precipitate is granular, and allow to stand in cool place overnight. Filter and wash free from Cl with cold  $\text{NH}_4\text{OH}$  (1+10). Ignite, and weigh as  $\text{Mg}_2\text{P}_2\text{O}_7$ . (If sample is excessively high in Mn, dissolve ignited precipitate in  $\text{HNO}_3$ , determine Mn according to 12.15, and correct  $\text{Mg}_2\text{P}_2\text{O}_7$  for  $\text{Mn}_2\text{P}_2\text{O}_7$ .) Report as percentage of Mg.

#### 12.15

#### MANGANESE (8)—OFFICIAL

To aliquot of soln A, 12.5, representing 0.2–0.5 g of ash, add 15 ml of  $\text{H}_2\text{SO}_4$  and evaporate to ca 30 ml. Add 5–10 ml of  $\text{HNO}_3$  and continue evaporation. (It is neither necessary nor advisable to evaporate until dense fumes appear, since the  $\text{Fe}_2(\text{SO}_4)_3$  then dissolves with difficulty.  $\text{HNO}_3$  may be present, but not  $\text{HCl}$ .) Add  $\text{H}_2\text{O}$ , a little at a time, heat until the Fe salts have dissolved, and dilute to ca

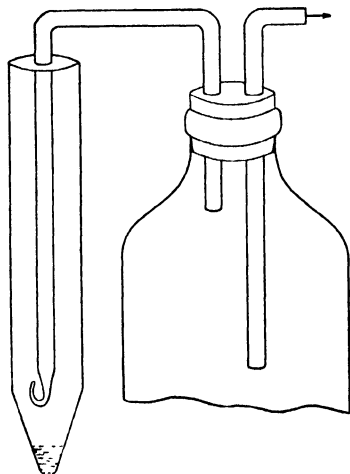


FIG. 15.—SUCTION DEVICE USED IN MICRO METHOD FOR DETERMINATION OF CALCIUM

150 ml. Add 0.3 g of  $\text{KIO}_4$ , or its equivalent in  $\text{HIO}_4$ , in small portions, boil a few minutes or until the color of the  $\text{KMnO}_4$  shows no further increase in intensity, and allow to cool.

Prepare standard as follows: To volume of  $\text{H}_2\text{O}$  equal to sample add 15 ml of  $\text{H}_2\text{SO}_4$  and sufficient pure  $\text{Fe}(\text{NO}_3)_3$ , free from Mn, to equal ca the quantity of Fe in sample. Add measured quantity of 0.1 N  $\text{KMnO}_4$  soln until color is slightly darker than sample, then 0.3 g of  $\text{KIO}_4$ , and boil a few minutes. When cool, transfer sample and standard to 250 ml flasks and dilute to mark with  $\text{H}_2\text{O}$ . If color is weak, it may be necessary to dilute to less than 250 ml. Compare colors in any standard colorimeter. (A photoelectric colorimeter equipped with a suitable light filter may be used.) Report results as percentage of Mn.

## 12.16

## SODIUM AND POTASSIUM—OFFICIAL

Moisten 1–10 g of sample with  $\text{H}_2\text{SO}_4$  (1 + 10), dry in oven, and ignite in muffle at a low red heat to destroy organic matter. Heat residue on steam bath with 2–5 ml of  $\text{HCl}$  and ca 50 ml of  $\text{H}_2\text{O}$ . Transfer to beaker and add  $\text{NH}_4\text{OH}$  dropwise until precipitate formed requires several seconds to dissolve, thus leaving soln but faintly acid. Heat nearly to boiling, and add  $\text{NH}_4\text{OH}$  to precipitate all the Fe, Al, etc. Boil in covered beaker ca 1 min.; remove, and if no  $\text{NH}_3$  is detected by smelling, continue addition, dropwise, until it can be detected. Do not allow precipitate to settle, but stir and pour on filter. Wash immediately with hot  $\text{H}_2\text{O}$ , using, to effect rapid filtration, a fine jet directed around edge of precipitate to cut it free from the paper. Wash precipitate several times, return to original beaker, dissolve with a few drops of  $\text{HCl}$ , and warm. Reprecipitate the Fe, Al, and  $\text{P}_2\text{O}_5$  with  $\text{NH}_4\text{OH}$  as directed above; filter and wash until free from Cl. Evaporate combined filtrates and washings to dryness, heat below redness until  $\text{NH}_4$  salts are expelled, and dissolve in hot  $\text{H}_2\text{O}$ . Add 5 ml of a saturated  $\text{Ba}(\text{OH})_2$  soln, heat to boiling, allow to settle a few minutes, and determine whether or not precipitation is complete by addition of more of the  $\text{Ba}(\text{OH})_2$  soln to a little of the clear liquid. When no further precipitate is produced, filter and wash thoroly with hot  $\text{H}_2\text{O}$ . Heat filtrate to boiling and add  $\text{NH}_4\text{OH}$  (1 + 4) and a 10%  $(\text{NH}_4)_2\text{CO}_3$  soln to complete precipitation of the Ba, Ca, etc. Let stand short time on water bath, filter, and wash precipitate thoroly with hot  $\text{H}_2\text{O}$ . Evaporate filtrate and washings to dryness, expel  $\text{NH}_4$  salts by heating below redness, treat with a little hot  $\text{H}_2\text{O}$ , and add a few drops of the dilute  $\text{NH}_4\text{OH}$ , 1 or 2 drops of the  $(\text{NH}_4)_2\text{CO}_3$  soln, and a few drops of a saturated  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  soln. Let stand a few minutes on water bath and set aside a few hours. Filter, evaporate to complete dryness on water bath, and heat at temp. not exceeding dull redness until all  $\text{NH}_4$  salts are expelled and residue is nearly or quite white. Dissolve in minimum quantity of  $\text{H}_2\text{O}$ , filter into weighed Pt dish, add a few drops of  $\text{HCl}$ , evaporate to dryness on water bath, heat at temp. not exceeding dull redness, cool in desiccator, and weigh as  $\text{KCl}$  plus  $\text{NaCl}$ . Repeat the heating until constant weight is obtained.

## POTASSIUM

## 12.17

*Platinic Chloride Method—Official*

Dissolve residue of mixed chlorides, 12.16, with a few ml of  $\text{H}_2\text{O}$ , acidify with a few drops of  $\text{HCl}$ , and add excess of  $\text{H}_2\text{PtCl}_6$  soln, 2.40(b). Evaporate on water bath to thick paste; treat residue repeatedly with 80% alcohol, decanting thru a weighed Gooch crucible or other form of filter; transfer precipitate to filter; and wash with the 80% alcohol until filtrate is colorless. Dry 30 min. at  $100^\circ$  and weigh.  $\text{K}_2\text{PtCl}_6 \times 0.1608 = \text{K}$ . If it is desired to determine the Na, calculate the K to  $\text{KCl}$  and subtract this from the  $\text{KCl} + \text{NaCl}$ , 12.16.

## 12.18

*Perchloric Acid Method (9)—Tentative*

Prepare material as directed in 12.16 until heavy metals have been removed and the two elements are in the form of chlorides. (Sulfates must be absent.) Add 3–5 ml of 60%  $\text{HClO}_4$ . Evaporate to dryness, dissolve in hot  $\text{H}_2\text{O}$ , and again evaporate to dryness. Heat to  $350^\circ$ , cool, and weigh if it is desired to obtain combined perchlorates. Add 10–20 ml of a mixture of anhydrous ethyl acetate and normal butanol in equal proportions by volume. Digest near b.p. several minutes. Decant into Gooch crucible. Wash once or twice by decantation with a few ml of the acetate-butanol mixture. Dissolve in minimum quantity of  $\text{H}_2\text{O}$ , evaporate to dryness, and extract as before. Filter, and wash several times with 1 ml of the acetate-butanol mixture. Dry in oven at  $110^\circ$  several minutes and heat at  $350^\circ$  15 min. Cool, and weigh.  $\text{KClO}_4 \times 0.2822 = \text{K}$ . Calculate Na by difference.

## 12.19

*Rapid Method for Potassium Only—Official*

Proceed as directed under 12.16 to "and if no  $\text{NH}_3$  is detected . . . until it can be detected." Add a few ml of saturated  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  soln, let stand a few hours, filter, and wash with hot  $\text{H}_2\text{O}$  until free from chlorides. Concentrate to small volume, transfer to Pt evaporating dish, evaporate to drive off excess  $\text{NH}_3$ , add 0.5 ml of  $\text{H}_2\text{SO}_4$  (1+1), evaporate, ignite by whirling dish over a free flame, and proceed as directed under 12.17.

## SODIUM ONLY (9)—TENTATIVE

## 12.20

## REAGENT

*Magnesium uranyl acetate soln:*

(a) *Crystallized uranyl acetate.*—To 85 g add 60 g of acetic acid and  $\text{H}_2\text{O}$  to make 1000 ml.

(b) *Crystallized magnesium acetate.*—To 500 g add 60 g of acetic acid and  $\text{H}_2\text{O}$  to make 1000 ml.

Heat (a) and (b) separately to ca  $70^\circ$  until all salts are dissolved. Mix two solns at this temp. and allow to cool to ca  $30^\circ$ . Place vessel containing mixed reagent in  $\text{H}_2\text{O}$  at  $20^\circ$ , and hold at this temp. 1–2 hours, or until the slight excess of salts is crystallized out. Filter reagent thru dry filter into dry bottle.

## 12.21

## DETERMINATION

Moisten 1–10 g of sample with  $\text{H}_2\text{SO}_4$  (1+10), dry in oven, and ignite in muffle at low red heat to destroy organic matter. Heat residue on steam bath with 2–5 ml of  $\text{HCl}$ , add ca 40 ml of  $\text{H}_2\text{O}$ , and heat to boiling. Add sufficient  $\text{CaCl}_2$  soln to precipitate all phosphates. Precipitate phosphates by making slightly alkaline with  $\text{NH}_4\text{OH}$ . Filter, and evaporate to 5 ml or less if no salts separate. Cool. Add 100 ml of the Mg uranyl acetate soln, place mixture in water bath at  $20^\circ$ , and either stir vigorously 45 min. or let stand 24 hours. Filter with suction and wash with alcohol saturated with the Na-Mg-uranyl acetate. Dry at  $105$ – $110^\circ$  30 min., cool, and weigh.  $\text{Weight of Na-Mg-uranyl acetate} \times 0.0153 = \text{Na}$ .

## COPPER (10)—TENTATIVE

## 12.22

## REAGENTS

(a) *Ammonium hydroxide soln.*—(1+1).

(b) *Carbamate soln.*—Dissolve 1 g of Na diethyldithiocarbamate in  $\text{H}_2\text{O}$  and dilute to 1 liter. Filter if necessary.

(c) *Carbon tetrachloride*.—Isoamyl alcohol may also be used.

(d) *Citric acid soln.*—15%. Dissolve 150 g of citric acid in  $H_2O$  and dilute to 1 liter.

(e) *Standard copper soln.*:

Soln 1.—Dissolve 0.7587 g of anhydrous  $CuSO_4$  (Merck's reagent quality 99.668% pure) in  $H_2O$  and dilute to 1 liter.

Soln 2.—Dilute 100 ml of Soln 1 to 1 liter. 1 ml contains .030 mg of Cu.

## 12.23

### DETERMINATION

Weigh samples of 1–20 g into porcelain crucibles and ignite in muffle furnace at dull red heat. Remove from furnace and cool. If sample remains very black, remove from muffle, cool, add  $H_2O$ , break up particles, dry, and reignite. Add 3–5 ml of  $HCl$  and boil for 1 min. Add a few ml of hot  $H_2O$  and filter into volumetric flask, usually of 200 ml capacity. Transfer insoluble residue to filter and wash with hot  $H_2O$  until free of  $Cl$ . Cool, make to volume, and mix. Pipet aliquot containing .015–.030 mg of Cu into a 125 ml separator and add 5 ml of the citric acid soln. Drop a small piece of litmus paper into separator, and neutralize the acid with the  $NH_4OH$ . Add 10 ml of the carbamate soln and 4 ml of  $CCl_4$  to separator and shake mixture vigorously for 2–3 min. Allow layers to separate, shake down the  $CCl_4$  from upper surface, dry stem of separator, and draw off  $CCl_4$  layer into dry 25 ml Erlenmeyer flask. Repeat extraction 3 more times, or until the  $CCl_4$  remains colorless. Draw off  $CCl_4$  each time and collect all extracts in same flask. Filter the  $CCl_4$  soln thru a small quantity of anhydrous  $Na_2SO_4$  into 25 ml volumetric flask. Wash filter paper several times with small portions of  $CCl_4$ , make to volume, and mix thoroughly. Determine per cent of light transmitted by each soln with photoelectric colorimeter equipped with 1 cm absorption cells and a light filter with maximum transmission near 4300–4400 Å. (No. 554, lantern-blue, Corning glass light filter is suitable.) Evaluate quantity of Cu present from a curve relating light transmission and concentration. Prepare curve by carrying known quantities of Cu and a blank soln thru procedure in exactly same manner as described for the unknowns, determining per cent of light transmitted by each soln, using the blank soln as standard of comparison, and plotting values thus obtained against respective concentrations.

### ZINC (11)—TENTATIVE

## 12.24

### REAGENTS

(All  $H_2O$  must be redistilled from Pyrex glass. All glassware must be rinsed with concentrated acid and then thoroly rinsed with Zn-free  $H_2O$ .)

(a) *Carbon tetrachloride*.—Use C. P. grade without purification. If Tech. grade is used, dry with anhydrous  $CaCl_2$  and redistil in presence of small quantity of  $CaO$ . (Used  $CCl_4$  may be reclaimed by distillation in presence of  $NaOH$  (1+100) containing small quantities of  $Na_2S_2O_3$ , followed by drying with anhydrous  $CaCl_2$  and fractional distillation in presence of small quantities of  $CaO$ .)

(b) *Standard zinc solns.*—(1) *Stock soln (1 mg of Zn/ml)*.—Place 0.25 g of pure Zn in 250 ml volumetric flask. Add ca 50 ml of  $H_2O$  and 1 ml of  $H_2SO_4$ , then heat on steam bath until all Zn is dissolved. Dilute to 250 ml and store in Pyrex vessel. (2) *Standard soln (10 micrograms of Zn/ml)*.—Dilute 10 ml of stock soln to 1 liter. Store in Pyrex vessel.

(c) *Ammonium hydroxide soln.*—Normal. With an all-Pyrex glass apparatus distil into  $H_2O$  one-half of a volume of  $NH_4OH$  and dilute to proper concentration. Store in glass-stoppered Pyrex vessel.

(d) *Hydrochloric acid*.—Normal. Displace  $HCl$  gas from  $HCl$  in a glass flask by



slow addition of equal volume of  $\text{H}_2\text{SO}_4$  by means of a dropping funnel that extends below surface of the  $\text{HCl}$ . Absorb displaced  $\text{HCl}$  gas by conducting it thru a delivery tube to surface of a volume of  $\text{H}_2\text{O}$ . (No heat is necessary.) Dilute to proper concentration. 150 ml of each of the acids will yield 1 liter of purified  $\text{HCl}$  soln of concentration greater than normal.

(e) *Diphenylthiocarbazone (dithizone) soln.*—Dissolve 0.20 g of dithizone in 500 ml of  $\text{CCl}_4$ , and filter soln to remove insoluble matter. Place soln in glass-stoppered bottle or large separator, add 2 liters of 0.02  $N$   $\text{NH}_4\text{OH}$  (40 ml of normal  $\text{NH}_4\text{OH}$  diluted to 2 liters), then shake to extract the dithizone into the aqueous phase. Separate phases, discard  $\text{CCl}_4$  phase, and extract ammoniacal soln of dithizone with 100 ml portions of  $\text{CCl}_4$  until the  $\text{CCl}_4$  extract is a pure green color. Discard the  $\text{CCl}_4$  phase after each extraction. Add 50 ml of  $\text{CCl}_4$  and 45 ml of normal  $\text{HCl}$  and shake to extract the dithizone into the  $\text{CCl}_4$ . Separate phases and discard aqueous phase. Dilute the  $\text{CCl}_4$  soln of dithizone to 2 liters with  $\text{CCl}_4$ . Store in a brown bottle in dark, cool place.

(f) *Ammonium citrate soln.*—0.5  $M$ . Dissolve 226 g of  $(\text{NH}_4)_2\text{HC}_6\text{H}_5\text{O}_7$  in 2 liters of  $\text{H}_2\text{O}$ . Add  $\text{NH}_4\text{OH}$  until soln has pH of 8.5–8.7 (80–85 ml). Add excess of the dithizone soln (orange-yellow coloration in aqueous phase after shaking and separation of phases), and extract with 100 ml portions of  $\text{CCl}_4$  until extract is a full green color. Add more dithizone if necessary. Separate aqueous phase from the  $\text{CCl}_4$ , and store in Pyrex vessel.

(g) *Carbamate reagent.*—Dissolve 0.25 g of Na diethyldithiocarbamate in  $\text{H}_2\text{O}$  and dilute to 100 ml with  $\text{H}_2\text{O}$ . Prepare a fresh soln just before use.

## 12.25

### PREPARATION OF SOLUTIONS

To reduce measuring out reagents and minimize errors due to variations in composition, prepare 3 solns in appropriate quantities from the reagents and store in Pyrex vessels, taking care to avoid loss of  $\text{NH}_3$  from solns A and B. Discard solns after they have been stored 6–8 weeks because the Zn increases slowly with storage. Determine a standard curve for each new set of reagents. The quantity of reagents designated and 2 liters of dithizone reagent are sufficient for 100 determinations.

(1) *Soln A.*—Dilute 1 liter of 0.5  $M$   $\text{NH}_4$  citrate and 140 ml of normal  $\text{NH}_4\text{OH}$  to 4 liters.

(2) *Soln B.*—Dilute 1 liter of 0.5  $M$   $\text{NH}_4$  citrate and 300 ml of normal  $\text{NH}_4\text{OH}$  to 4.5 liters. Just before using add 1 volume of the freshly prepared carbamate reagent to 9 volumes of the  $\text{NH}_3$ - $\text{NH}_4$  citrate soln to obtain volume of soln B immediately required.

(h) *Hydrochloric acid.*—0.02  $N$ . Dilute 100 ml of normal  $\text{HCl}$  to 5 liters.

NOTE: If reagents purified from Zn have been prepared, they can be used to test chemicals for Zn. Certain lots of  $\text{NH}_4\text{OH}$  and  $\text{HCl}$  are sufficiently free of Zn to be used in this method without purification.

## 12.26

### ASHING

Ash 5 g sample of the finely ground, air-dry plant material in a Pt dish in an electric muffle at 500–550°. Include a blank determination. Moisten ash with a little  $\text{H}_2\text{O}$ , then add 10 ml of the normal  $\text{HCl}$  (more if necessary) and heat on steam bath until all substances soluble in  $\text{HCl}$  are brought into soln. Add 5–10 ml of hot  $\text{H}_2\text{O}$ . Filter off insoluble matter on a 7 cm filter paper (Whatman No. 42 or equivalent) that has been washed with two 5 ml portions of hot normal  $\text{HCl}$ , then washed with hot  $\text{H}_2\text{O}$  until free of  $\text{HCl}$ , and collect filtrate in 100 ml volumetric flask. Wash filter with hot  $\text{H}_2\text{O}$  until washings are no longer acid to methyl red. Add drop of methyl red indicator to filtrate in 100 ml flask, neutralize with normal  $\text{NH}_4\text{OH}$  and add 4

ml of normal HCl. Allow contents of the flask to cool, then make to volume with  $H_2O$ .

## 12.27

## FIRST EXTRACTION

(Separation of dithizone complex-forming metals from ash soln)

Pipet aliquot of the ash soln containing not more than 30 micrograms of Zn into a 125 ml Squibb separator. Add 1 ml of 0.2 *N* HCl for each 5 ml of ash soln less than 10 ml taken or 1 ml of 0.2 *N*  $NH_4OH$  for each 5 ml over 10 ml taken. (A 10 ml aliquot has usually been found satisfactory in the analysis of plant materials.) Add 40 ml of soln A and 10 ml of the dithizone reagent. Shake vigorously for 30 seconds to extract from the aqueous phase the Zn and other dithizone complex-forming metals that may be present, then allow layers to separate. At this point an excess of dithizone (indicated by orange or yellow-orange coloration of aqueous phase) must be present. If excess dithizone is not present, add more of the reagent until after shaking an excess is indicated. Shake down the drop of  $CCl_4$  extract from surface, and draw off the  $CCl_4$  extract into second separator as completely as possible without allowing any of aqueous layer to enter stopcock bore. Rinse down the  $CCl_4$  extract from surface of the aqueous layer with 1–2 ml portion of clear  $CCl_4$ , then run off this  $CCl_4$  into second separator without permitting aqueous phase to enter stopcock bore. Repeat this rinsing process as many times as necessary to completely flush extract into second separator. Add 5 ml of clear  $CCl_4$  to first separator, shake 30 seconds, then allow layers to separate. (The  $CCl_4$  layer at this point will have clear green color if the metals that form dithizone complexes have been completely extracted from aqueous phase by previous extraction.) Run off  $CCl_4$  layer into second separator, then flush down extract from surface and out of separator as directed previously. If last extract does not possess a distinct clear green color, repeat the extraction with 5 ml portion of clear  $CCl_4$  and the flushing out process until complete extraction of the dithizone complex-forming metals is assured, then discard aqueous phase.

## 12.28

## SECOND EXTRACTION

(Separation of Cu by extraction of Zn into 0.02 *N* HCl)

Pipet 50 ml of 0.02 *N* HCl into the separator containing the  $CCl_4$  soln of meta-dithizonates. Shake vigorously for 1.5 min., then allow layers to separate. Shake down the drop from surface of aqueous phase, and as completely as possible run off the  $CCl_4$  phase that contains all the Cu as dithizonate, without allowing any of aqueous phase, which contains all the Zn, to enter the stopcock bore. Rinse down  $CCl_4$  extract from surface of aqueous phase and rinse out stopcock bore with 1–2 ml portions of clear  $CCl_4$  (same as in the first extraction) until all traces of green dithizone have been washed out of separator. Shake down the drop of  $CCl_4$  from surface of aqueous phase, and run off  $CCl_4$  as completely as possible without allowing any aqueous phase to enter stopcock bore. Remove stopper from separator and lay it across the neck until the small quantity of  $CCl_4$  on surface of aqueous phase has evaporated.

## 12.29

## FINAL EXTRACTION

(Extraction of Zn in presence of carbamate reagent)

Pipet 50 ml of soln B and 10 ml of dithizone reagent into 50 ml of 0.02 *N* HCl soln containing the Zn. Shake for 1 min, then allow phases to separate. Flush out stopcock and stem of separator with ca 1 ml of the  $CCl_4$  extract, then collect re-

mainder in test tube. Pipet 5 ml of the extract into 25 ml volumetric flask, dilute to mark with clear  $\text{CCl}_4$ , and then determine percentage of light transmission of diluted soln with a photoelectric colorimeter, equipped with a Sextant Green (Corning No. 401) filter, or equivalent. (CAUTION: Final extract should be protected from sunlight as much as possible and read in photoelectric colorimeter within 2 hours.)

Determine the amount of Zn present in the aliquot from curve relating per cent transmission and concentration, correct for Zn in blank, and calculate per cent of Zn in sample.

**12.30****STANDARD CURVE**

Obtain data for standard curve by determining per cent transmission values for each of a series of solns containing known quantities of Zn. To prepare these Zn solns, place 0, 5, 10, 15, 20, 25, 30, and 35 ml of the standard Zn soln containing 10 micrograms of Zn per ml in 100 ml volumetric flasks. To each flask add 1 drop of methyl red indicator, then normal  $\text{NH}_4\text{OH}$  until neutral, then 4 ml of normal  $\text{HCl}$ , and make to volume. Proceed exactly as directed for the ash solns, beginning with the first extraction, and using 10 ml aliquots of each of Zn solns. (The 10 ml aliquots contain 0, 5, 10, 15, 20, 25, 30, and 35 micrograms of Zn, respectively.) Construct the standard curve by plotting micrograms of Zn against per cent light transmission on semi-log paper.

**ARSENIC—TENTATIVE****12.31****PREPARATION OF SOLUTION.—See 29.3****12.32****DETERMINATION**

Proceed as directed under 29.4 and 29.5, or take aliquot and determine as directed under 6.12, beginning "add 3 ml of  $\text{H}_2\text{SO}_4$ ."

**SULFUR***Sodium Peroxide Method (12)—Official***12.33****PREPARATION OF SOLUTION**

Place 1.5–2.5 g of material in Ni crucible of ca 100 ml capacity and add 5 g of anhydrous  $\text{Na}_2\text{CO}_3$ . Mix thoroly, using Ni or Pt rod, and moisten with ca 2 ml of  $\text{H}_2\text{O}$ . Add  $\text{Na}_2\text{O}_2$ , ca 0.5 g at a time, thoroly mixing charge after each addition, and continue until mixture becomes nearly dry and quite granular (ca 5 g of  $\text{Na}_2\text{O}_2$  required). Place crucible over a S-free flame or electric hot plate and heat carefully, with occasional stirring until contents are fused. (If material ignites, determination is worthless.) After fusion, remove crucible, allow to cool somewhat, and cover hardened mass with more of the  $\text{Na}_2\text{O}_2$  to depth of ca 0.5 cm. Heat gradually and finally with full flame until fusion again takes place, rotating crucible from time to time in order to bring any particles adhering to sides into contact with oxidizing material. Continue heating 10 min. after fusion is complete. Cool somewhat, place warm crucible and contents in 600 ml beaker, and carefully add ca 100 ml of  $\text{H}_2\text{O}$ . After initial violent action has ceased, wash material out of crucible, make slightly acid with  $\text{HCl}$  (adding small portions at a time), transfer to 500 ml flask, cool, and dilute to volume. Filter, and determine sulfates in an aliquot of filtrate as directed under 12.34.

**12.34****DETERMINATION**

Add  $\text{H}_2\text{O}$  to make aliquot to ca 200 ml and add  $\text{HCl}$  to make ca 0.5 ml of free acid present. Heat to boiling and add 10 ml of 10%  $\text{BaCl}_2$  soln dropwise with constant

stirring. Continue boiling ca 5 min. and allow to stand 5 hours or longer in warm place. Decant liquid thru ashless filter or ignited and weighed Gooch crucible, treat precipitate with 15–20 ml of boiling  $H_2O$ , transfer to filter, and wash with boiling  $H_2O$  until filtrate is free from Cl. Dry precipitate and filter, ignite, and weigh as  $BaSO_4$ . Multiply result by factor 0.1374 and report as percentage of S.

*Magnesium Nitrate Method (13)—Official*

**12.35**

**PREPARATION OF SOLUTION**

Weigh 1 g of material into large porcelain or Sillimanite crucible. Add 7.5 ml of  $Mg(NO_3)_2$  soln, 2.7(e), taking care that all material is brought in contact with soln. (As it is important that sufficient  $Mg(NO_3)_2$  soln be added to insure complete oxidation and fixation of S present, for larger samples and for samples with a high S content a proportionally greater amount of this soln must be used.) Heat on electric hot plate ( $180^\circ$ ) until no further action takes place. Transfer crucible while hot to electric muffle and allow it to remain at low heat (muffle must not show any red) until charge is thoroly oxidized. (No black particles should remain. It may be necessary to break up charge and return to muffle.) Remove crucible from muffle and allow to cool. Add  $H_2O$ , then HCl in excess. Bring soln to boil, filter, and wash thoroly. If preferred, transfer soln to 250 ml volumetric flask before filtering and make to mark with  $H_2O$ .

**12.36**

**DETERMINATION**

Dilute entire filtered soln, 12.35, to 200 ml or take an aliquot of 100 ml of the measured volume, make to 200 ml, and proceed as directed under 12.34.

**PHOSPHORUS (14)**

**12.37**

*Macro Method—Official*

(a) *For samples exceedingly high in P and low in Ca and Mg such as certain seeds, grains, etc.*—Prepare as directed under 12.35, or evaporate filtrate and washings from S determination, 12.34, to 50 ml and proceed as directed under 2.9 or 2.12.

(b) *For all other samples.*—Take 50 ml aliquot of soln A, 12.5, and proceed as directed under 2.9 or 2.12.

*Micro Method (15)—Official*

**12.38**

**REAGENTS**

(a) *Standard potassium dihydrogen phosphate soln.*—Dissolve 0.4394 g of pure dry  $KH_2PO_4$  in  $H_2O$  and make up to liter. 50 ml of this soln diluted to 200 ml gives a standard of which 2 ml = 0.05 mg of P.

(b) *Ammonium molybdate soln.*—Dissolve 25 g of  $NH_4$  molybdate in 300 ml of  $H_2O$ . Dilute 75 ml of  $H_2SO_4$  to 200 ml and add to the  $NH_4$  molybdate soln.

(c) *Hydroquinone soln.*—Dissolve 0.5 g of hydroquinone in 100 ml of  $H_2O$ , and add one drop of  $H_2SO_4$  to retard oxidation.

(d) *Sodium sulfite soln.*—Dissolve 200 g of  $Na_2SO_3$  in  $H_2O$ , make up to a liter, and filter. Either keep this soln well-stoppered or prepare fresh each time.

(e) *Magnesium nitrate soln.*—Dissolve 160 g of  $MgO$  in  $HNO_3$  (1+1), avoiding excess of the acid; add a little  $MgO$  in excess, boil, filter from the excess  $MgO$ ,  $Fe_2O_3$ , etc., and dilute to 1 liter.

**12.39**

**PREPARATION OF SOLUTION**

To 1 or 2 g of substance in small Sillimanite crucible add 1 ml of the  $Mg(NO_3)_2$  soln, and place on steam bath. After a few minutes cautiously add a few drops of

HCl, taking care that formation of gas bubbles does not push portions of sample over edge of crucible. Make 2 or 3 further additions of a few drops of HCl while sample is on bath so that as it approaches dryness there is a tendency for it to char. If contents of crucible become so viscous that no further drying may be obtained on bath complete drying on hot plate, put on crucible cover, transfer to cold muffle, and ignite at dull red heat 6 hours, or until an even grey ash is obtained. (It may be necessary to cool crucible, dissolve ash in a little  $H_2O$  or alcoholic glycerol, evaporate to dryness, and return uncovered to muffle for 4–5 hours longer.) Cool, take up with HCl (1+4), and transfer to 100 ml beaker. Add 5 ml of HCl and evaporate to dryness on steam bath to dehydrate  $SiO_2$ . Moisten residue with 2 ml of HCl, add ca 50 ml of  $H_2O$ , heat a few minutes on bath, transfer to 100 ml volumetric flask, cool immediately, make to volume, and filter, discarding first portion of filtrate.

**12.40****DETERMINATION**

To 5 ml aliquot of filtrate in 10 ml volumetric flask add 1 ml of the  $NH_4$  molybdate soln, rotate flask to mix, and allow to stand a few moments. Add 1 ml of the hydroquinone soln, again rotate flask, and add 1 ml of the  $Na_2SO_3$  soln. (These last 3 additions may be made with a Mohr pipet.) Make to volume with  $H_2O$ , stopper mouth of flask with thumb or forefinger, and shake to mix contents thoroly. Allow to stand 30 min. and compare immediately in a colorimeter with 2 ml of the standard  $KH_2PO_4$  soln treated simultaneously and in identical manner. (With either unknown or standard set at 25.0 mm, readings within 10 mm, i.e., a range of 20 mm, are accurate. If concentration of P in unknown set is outside this range, it may be brought nearer to that of standard by diluting filtrate, ashing a smaller or larger sample, making filtrate to smaller or larger volume, or using smaller aliquot. Photoelectric colorimeter equipped with suitable light filter may be used instead of visual instrument.) Report as percentage of P.

**CHLORINE (16)**

(If bromides or iodides are present in significant quantities results must be corrected accordingly.)

**12.41****PREPARATION OF SOLUTION—OFFICIAL**

First verify complete retention of Cl in each kind of material by trial since losses can occur, especially with samples high in carbohydrates, if insufficient  $Na_2CO_3$  is present during ignition, or in any case if excessive temps. are used.

Moisten 5 g of substance in Pt dish with 20 ml of a 5%  $Na_2CO_3$  soln, evaporate to dryness, and ignite as thoroly as possible at a temp. not exceeding dull redness. Extract with hot  $H_2O$ , filter, and wash. Return residue to Pt dish and ignite to ash; dissolve in  $HNO_3$  (1+4), filter from any insoluble residue, wash thoroly, and add this soln to  $H_2O$  extract.

**12.42****Gravimetric Method—Official**

To prepared soln, 12.41, add 10%  $AgNO_3$  soln, avoiding more than slight excess. Heat to boiling, protect from light, and allow to stand until precipitate is coagulated. Filter on weighed Gooch crucible, previously heated to 140–150°, and wash with hot  $H_2O$ , testing filtrate to prove excess of  $AgNO_3$ . Dry the  $AgCl$  at 140–150°, cool, and weigh. Report as percentage of Cl.

**Volumetric Method I (17)—Official**

(Limit of accuracy of this titration is considered to be  $\pm 0.2$  mg of Cl, hence an accuracy of 1.0% would require samples containing not less than 20 mg.)

## 12.43

## REAGENTS

(a) *Silver nitrate soln.*—Adjust to exact 0.1 *N* strength by standardizing against a 0.1 *N* NaCl soln containing 5.846 g of pure NaCl/liter.

(b) *Ammonium or potassium thiocyanate.*—0.1 *N*. Adjust by titrating against the 0.1 *N* AgNO<sub>3</sub>.

(c) *Ferric indicator.*—A saturated soln of FeNH<sub>4</sub>(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O.

(d) *Nitric acid.*—Free from lower oxides of N by diluting the usual pure acid with ca ½ volume of H<sub>2</sub>O, and boiling until perfectly colorless.

## 12.44

## DETERMINATION

To the prepared soln, 12.41, add a known volume of the AgNO<sub>3</sub> soln in slight excess. Stir well, filter, and wash AgCl precipitate thoroly. To combined filtrate and washings add 5 ml of the ferric indicator and a few ml of the HNO<sub>3</sub> and titrate excess of Ag with the thiocyanate until a permanent light brown color appears. From number of ml of AgNO<sub>3</sub> used, calculate quantity of Cl. 1 ml of 0.1 *N* AgNO<sub>3</sub> = 0.00355 g of Cl.

*Volumetric Method II (18)—Official*

## 12.45

## REAGENTS

(a) *Standard potassium iodide soln.*—Weigh out 4.6822 g of pure (A.C.S. spec.) KI, dried to constant weight at 105–150°, dissolve in H<sub>2</sub>O, and dilute to 1 liter. 1 ml = 1 mg of Cl.

(b) *Silver nitrate soln.*—Approximately 0.3 *N*. Dissolve 48 g of AgNO<sub>3</sub> in H<sub>2</sub>O, filter, and dilute to 1 liter. 1 ml = 10 mg of Cl (approximately).

(c) *Standard silver nitrate soln.*—Dilute 100 ml of reagent (b) to ca 900 ml and adjust by standardizing against reagent (a) so that 1 ml = 1 mg of Cl.

(d) *Chlorine-free starch indicator.*—For each 100 ml of final soln take 2.5 g of soluble starch and make to a paste with cold H<sub>2</sub>O. After stirring out lumps, add 25–50 ml more cold H<sub>2</sub>O and stir or shake 5 min. Centrifuge, decant, and discard liquid. Repeat extraction 3 times and finally transfer residue to flask containing proper quantity of boiling H<sub>2</sub>O. Stir again, allow to come to boil, cover with small beaker, and cool under tap, shaking occasionally.

(e) *Dilute sulfuric acid soln.*—Add 35 ml of H<sub>2</sub>SO<sub>4</sub> to each liter of H<sub>2</sub>O, boil 5–10 min., and cool to room temp.

(f) *Iodine soln.*—Shake a large excess of I crystals in glass-stoppered bottle nearly filled with reagent (e). Decant, and discard soln. Repeat process but decant soln into glass-stoppered bottle. Test soln for presence of iodides by adding to a 25 ml portion an equal volume of reagent (e) and 5 ml of reagent (d). If no blue color appears within 5 min., iodides may be considered absent and soln is ready for use.

(g) *Potassium permanganate.*—Dissolve 60 g of KMnO<sub>4</sub> in 400 ml of warm H<sub>2</sub>O (ca 50°) and dilute to 1 liter.

(h) *Potassium sulfate—copper sulfate mixture.*—Thoroly mix 16 parts of K<sub>2</sub>SO<sub>4</sub> with 1 part of CuSO<sub>4</sub>·5H<sub>2</sub>O.

(i) *Wash soln.*—Mix 980 ml of H<sub>2</sub>O with 20 ml of HNO<sub>3</sub>.

## 12.46

## DETERMINATION

Weigh into beaker such a quantity of sample as is expected to contain 10–40 mg of Cl. (If more than 4 g is taken, use proportionately more HNO<sub>3</sub> and KMnO<sub>4</sub> soln.) Add 10 ml of the 0.3 *N* AgNO<sub>3</sub> soln and stir until sample is thoroly soaked with the soln, adding a little H<sub>2</sub>O or warming if necessary. Add 25 ml of HNO<sub>3</sub>, stir, add 5 ml of the KMnO<sub>4</sub> soln, and stir until frothing ceases. Place mixture in water

bath or on hot plate to keep it just below boiling. Stir, and wash down sides of beaker at intervals with least possible quantity of  $H_2O$ . After 20 min., or when there appears to be no further action on sample, add more of the  $KMnO_4$  soln, a little at a time, until color begins to fade slowly. Dilute to ca 125 ml with boiling  $H_2O$  and heat 10 min. longer. (Beaker may stand in bath or on hot plate until ready to filter.) Filter while hot thru Whatman No. 5, or similar paper, with suction as follows: Place disk of 30-mesh stainless steel wire gauze or of No. 40 filter cloth in bottom of 3" Hirsch funnel. Fold 9 cm paper over bottom of No. 11 rubber stopper, shaping it to funnel by making 9-10 folds up side of stopper. Place paper in funnel and apply strong suction. Wet paper and keep it wet while fitting it into funnel so as to avoid double thicknesses of paper. Thoroughly wash paper, first with  $H_2O$ , then with the wash soln. Discard washings and rinse out flask. Pour supernatant liquid thru filter and transfer precipitate and sample residue to filter. If filtrate is not turbid, or if it is only slightly opalescent, wash precipitate thoroughly, applying wash soln very gently, but maintaining strong suction on filter. If combined filtrate and washings are clear, test them for Ag. If turbid, re-heat and pass thru filter, repeating until clear, and finally wash as directed above. If filtrate does not give a definite test for Ag, repeat determination on fresh but smaller portion of sample. Place filter paper and contents in Kjeldahl flask and add such quantities of mixture of  $CuSO_4$  and  $K_2SO_4$  and of  $H_2SO_4$  as would be appropriate for a protein determination on same kind and amount of material and digest in similar manner. (For 2 g of grass, 8 g of the sulfate mixture and 20 ml of acid are enough.) When digest is cool, add 175 ml of  $H_2O$ , boil 5-10 min., and cool to room temp. Titrate the  $Ag_2SO_4$  in the Kjeldahl flask with the standard KI, using 5 ml of starch and 30 ml of the I soln. (Latter is added just before titration.) Rinse neck of flask after each addition of KI when near end point and titrate until blue color persists after shaking. If less than 30 mg of Cl is present, add the starch and I soln at beginning. If a larger but unknown amount is present, add 2 ml of starch and 10 ml of I soln at beginning and titrate until approach of end point is seen. Shake vigorously to coagulate precipitate, add remainder of starch and I, and proceed to end point. If a known large amount is present, titrate to within 2 ml of end point, shake as above, add indicator reagents, and continue titration. If end point is over-run, add 5 ml of the standard  $AgNO_3$  and titrate again.

Blank determinations are not necessary after reagents have been tested. If blanks made by using pure sugar as a sample exceed 0.05 mg, examine filter paper and various reagents carefully.

## 12.47

### IODINE (19)—TENTATIVE

Weigh 50-100 g of finely ground, air-dried plant material; transfer to dry, clean, porcelain dish, and thoroughly mix with 10 g of finely pulverized  $CaO$  and 10 g of finely pulverized  $CuO$ . Transfer mixture onto curved pieces of nichrome wire gauze placed in U-shaped boat made from sheet Ni. Cut and bend edges of gauze so that they fit against walls of boat near bottom. Spread sample in uniform layer on top of gauze to allow air passing thru combustion tube, Fig. 16, to flow beneath and above sample during combustion, and to shorten time required for complete combustion. Place boat containing sample in combustion tube and fill wash bottles and absorption tube to proper volume with 5%  $K_2CO_3$  soln. Make system air tight, connect exit end of condenser of absorption tube to suction pump, and start current of air flowing at moderate rate thru combustion tube. Connect electric current to electric combustion furnace, and when this portion of combustion tube attains dull red heat, light first gas burner at left on gas furnace, turn to height that will heat left end of large com-

bustion tube to dull red, and ignite sample at this point. Turn on additional burners in consecutive order at proper intervals to keep sample burning slowly, somewhat in manner of a lighted cigar. (A few trials will be necessary to develop proper technic of combustion to keep sample burning slowly with production of as little smoke as

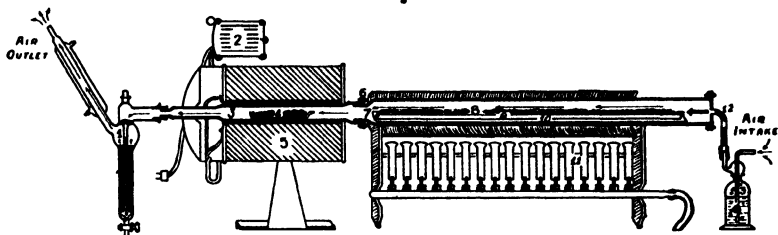


FIG. 16.—APPARATUS FOR DETERMINATION OF IODINE

(1) Glass absorber containing glass beads and 5%  $K_2CO_3$  soln; (2) rheostat; (3) catalyst tube; (4) boat containing catalyst of Cu turnings or coil of Cu wire; (5) electric furnace; (6) connection between sections 3 and 7 of combustion tube; (7) main combustion tube; (8) sample of plant material; (9) nichrome wire gauze for supporting sample of plant material; (10) boat made from sheet Ni; (11) gas combustion furnace; (12) intake for air; (13) wash bottle containing 10%  $K_2CO_3$  soln for removing I from air intake.

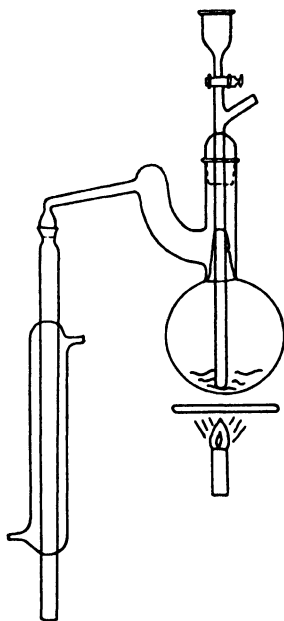


FIG. 17.—DISTILLATION APPARATUS

possible.) When current of air and heat from gas burners are properly adjusted, burn sample at such a rate that no smoke will pass unburned over the red-hot Cu catalyst and appear in glass absorption tube. (Presence of smoke or tar substances condensing in absorption tube indicates that flow of air and temp. are not properly regulated.)



After combustion is complete, transfer absorbing soln to modified 300 ml Claisen flask, Fig. 17, having ground-glass stoppers and connections. Also wash residue of ash into same flask.

Fig. 17 shows arrangement for distillation. End of tube leading from condenser goes to bottom of 50 ml extraction flask which contains just sufficient  $H_2O$  to cover end of condenser tube, 0.2 ml of 3%  $H_2SO_4$ , and 0.2 ml of 10%  $NaHSO_3$  soln. To contents of Claisen flask add quickly 2 ml of 50%  $H_2SO_4$ , 1 drop of 10%  $Fe_2(SO_4)_3$  soln and 2 ml of 3%  $H_2O_2$  soln (superoxol diluted to make 3% soln). If necessary add more acid to make soln definitely acid, as indicated by presence or absence of  $Fe(OH)_3$ . Add a glass bead to prevent bumping. Immediately close flask with stopper and dropping funnel, outlet of which has been drawn to point. Vigorously boil contents of flask over microburner, taking care, however, to avoid flooding of side arm of Claisen flask. Add 1 or 2 additional 2 ml portions of the 3%  $H_2O_2$  soln thru dropping funnel during distillation. Discontinue heating during these additions to avoid carrying  $H_2O_2$  over with distillate. Discontinue distillation when volume in Claisen flask is reduced to ca 5 ml or when sulfates begin to crystallize on side of flask.

Place extraction flask on wire gauze with asbestos center and boil contents gently for 2 min. to expel  $CO_2$  and  $SO_2$ , using glass bead to prevent bumping. Immediately make soln alkaline to litmus paper by addition of 10%  $KOH$  soln (ca 3 drops). Carefully boil down soln to volume of 5–6 ml, add 1 drop of methyl orange, and neutralize soln by addition of 3%  $H_2SO_4$ . Then add 2 drops of 3%  $H_2SO_4$  in excess and 5 drops of  $Br-H_2O$ , which should cause soln upon shaking to turn yellow immediately. Then boil down very cautiously to ca 2 ml and cool on ice (should require 3–5 min. to completely remove excess  $Br$  with minimum loss of  $I$ ). Add 1 drop of 1% starch soln and 2 drops of 1%  $KI$  soln and titrate with 0.001  $N$   $Na_2SO_3$  delivered from 0.2 ml pipet graduated to 0.001 ml. Calculate the  $I$ .

**12.48****SELENIUM (#0)**

(Applicable to materials containing over 2 p.p.m. of  $Se$ )

Grind air-dried sample and carefully prepare uniform subsample. Prepare mixture of 50 ml of  $H_2SO_4$  and 100 ml of 68%  $HNO_3$  in 600 ml beaker. Add 5 g of powdered sample to acid mixture slowly, with stirring, restricting temp. of mixture to not above  $80^\circ$ . When first vigorous reaction is over, warm gently with occasional stirring until fumes of  $NO_2$  cease to evolve. Warm at temp. not to exceed  $120^\circ$  until slight darkening of liquid begins to appear. Transfer liquid to distillation apparatus and proceed as directed in 1.26.

**SUGARS (#1)****12.49****PREPARATION OF SOLUTION—OFFICIAL, FIRST ACTION**

(a) *Extraction*.—Prepare sample as described under 12.2(b). Pour alcoholic soln thru filter paper or extraction thimble, catching filtrate in volumetric flask. Transfer insoluble material to beaker, cover with 80% alcohol, warm on steam bath 1 hour, allow to cool, and again pour the alcoholic soln thru same filter. If second filtrate is highly colored, repeat extraction. Transfer residue to filter, allow to drain, and dry. Grind residue so that all particles will pass thru a 1 mm sieve, transfer to extraction thimble, and extract 12 hours in Soxhlet apparatus with 80% alcohol. Dry residue and save for starch determination. Combine alcoholic filtrates and make to volume at a definite temp. with 80% alcohol.

(b) *Clearing*.—Place an aliquot of the alcoholic extract in beaker on steam bath and drive off alcohol. Avoid evaporation to dryness by adding  $H_2O$  if necessary. When odor of alcohol has disappeared from sample, add ca 100 ml of  $H_2O$  and heat to  $80^\circ$  to soften gummy precipitates and break up insoluble masses. Cool to room temp. and proceed as directed under (1) or (2).

(1) Transfer soln to volumetric flask and rinse beaker thoroly with  $H_2O$ , adding rinsings to contents of flask. Add enough saturated neutral Pb acetate soln to produce a flocculent precipitate, shake thoroly, and allow to stand 15 min. Test supernatant liquid with a few drops of saturated Pb acetate soln. If more precipitate forms, shake, and allow to stand again; if no further precipitate forms, dilute to mark with  $H_2O$ , mix thoroly, and filter thru dry filter. Add sufficient solid Na oxalate to filtrate to precipitate all the Pb, and refilter thru dry paper. Test filtrate for presence of Pb with a little solid Na oxalate.

(2) Add double minimum amount of saturated neutral Pb acetate soln that is required to cause complete precipitation, as found by testing a portion of supernatant liquid with a few drops of dilute Na oxalate soln. After allowing mixture to stand a few minutes only, filter into beaker to which has been added an estimated excess of Na oxalate crystals. Allow Pb precipitate to drain on filter and wash with cold  $H_2O$  until filtrate no longer gives a precipitate in the oxalate soln. Excess of oxalate must be assured by testing with a drop of Pb acetate soln. Filter off and wash precipitated Pb oxalate, catching filtrate and washings in volumetric flask. Dilute to mark with  $H_2O$  and mix.

#### REDUCING SUGARS

12.50 *Munson and Walker General Method—Tentative.—See 34.39*

*Quisumbing and Thomas Method (22)*

12.51

#### REAGENTS

(a) *Copper sulfate soln*.—Wash crystals of  $CuSO_4 \cdot 5H_2O$  free from dust, etc., with  $H_2O$ , dissolve in hot  $H_2O$  to make a saturated soln, and filter. Determine Cu electrolytically and dilute soln so that 25 ml will contain 525 mg of Cu (41.2 g of  $CuSO_4 \cdot 5H_2O$  in 500 ml of soln).

(b) *Alkaline tartrate soln*.—Prepare saturated soln of NaOH (purified by alcohol) and let stand several days, until insoluble carbonates and other impurities have settled out. Siphon off clear soln and establish its alkalinity by titration with standard acid. Dissolve 173 g of highest purity Rochelle salt in  $H_2O$  in 500 ml graduated flask and add calculated quantity of NaOH soln so that 500 ml of this alkaline tartrate soln will contain exactly 65 g of NaOH. Make to mark with  $H_2O$ .

12.52

#### DETERMINATION

Measure exactly 25 ml each of the  $CuSO_4$  and alkaline tartrate solns into 400 ml Pyrex or Bohemian glass beaker, the diameter of which is ca 9 cm. Add 50 ml of sugar soln containing preferably 50–150 mg of sugar. Cover beaker with watch-glass and place in water bath maintained at  $80^\circ$ . After digesting exactly 30 min., filter the  $Cu_2O$  by suction thru mat of asbestos in Gooch crucible. Wash precipitate with  $H_2O$ . Determine Cu by one of methods below. Calculate weight of sugar from tables of Quisumbing and Thomas, 44.21.

(a) *Direct Weighing of Cuprous Oxide—(Official, First Action).—See 34.40.*

(b) *Volumetric Permanganate Method (23)—(Tentative).*—With aid of stirring rod transfer asbestos mat and  $Cu_2O$  back into beaker in which reduction took place. Rinse inside of crucible and lip of beaker with 10 ml of a soln of 240.9 g of  $FeNH_4$

( $\text{SO}_4$ )<sub>2</sub>·12H<sub>2</sub>O and 200 ml of H<sub>2</sub>SO<sub>4</sub> dissolved in H<sub>2</sub>O and made up to 1 liter. Cool the dilute H<sub>2</sub>SO<sub>4</sub> before adding the salt. Receive rinsings in beaker containing the Cu<sub>2</sub>O. Holding crucible over beaker, stir contents of beaker thoroly with stirring rod until the Cu<sub>2</sub>O has gone into soln. Wash crucible with ca 25 ml of hot H<sub>2</sub>O (80°), receiving washings in beaker. Stir contents of beaker and then raise beaker to see if any undissolved particles of Cu<sub>2</sub>O are resting on bottom. If so, press out each one with point of stirring rod until all have gone into soln. Add ca 125 ml more hot H<sub>2</sub>O. Add 1 drop of a soln of 0.15 g of orthophenanthroline monohydrate and 0.07 g of FeSO<sub>4</sub> in 10 ml of H<sub>2</sub>O. Titrate at once with continual stirring with 0.05 N KMnO<sub>4</sub>. (In a long titration it is best to add indicator just before end point is reached.) Standardize the KMnO<sub>4</sub> as directed in 43.18.

## 12.53

## SUCROSE—TENTATIVE

(a) *Hydrochloric Acid Inversion*

Using aliquot of cleared soln obtained in 12.49, proceed as directed under 27.32.

(b) *Invertase Inversion*

(1) *For plants giving an end point of hydrolysis within 2 hours.*—Pipet aliquot of cleared soln obtained in 12.49 into beaker in which reduction is to take place. Make slightly acid to methyl red with acetic acid. Add 3 drops of 1% soln of Wallerstein's red label invertase. Let mixture stand at room temp. 2 hours. Add reagents as directed in 12.52 or in 34.38, and determine reducing power. Calculate results as invert sugar. Deduct reducing power of original soln, also expressed as invert sugar, and multiply difference by factor 0.95.

(2) *For plants giving a slower end point of hydrolysis.*—Place aliquot of soln obtained in 12.49 in small volumetric flask. Make slightly acid to methyl red with acetic acid. Add 3 drops of 1% soln of Wallerstein red label invertase and a few drops of toluene. Stopper flask and let stand overnight or longer at room temp. Dilute to mark with H<sub>2</sub>O and use aliquot for reducing power as directed above. Results may include some other carbohydrates slowly hydrolyzed by invertase.

## 12.54

## ETHER EXTRACT—TENTATIVE.—See 27.22

## 12.55

## CRUDE FIBER—TENTATIVE.—See 27.27

## 12.56

## TOTAL NITROGEN.—See 2.27, 2.28

## 12.57

## ORGANIC AND AMMONIACAL NITROGEN—See 2.24, 2.25, 2.26

## AMMONIA IN TOBACCO (24)—TENTATIVE

## 12.58

## REAGENTS

(a) *Ammonium sulfate stock soln.*—Dissolve 2.358 g of pure salt in H<sub>2</sub>O and make up to 1000 ml. 2 ml = 1.0 mg of N. Preserve by adding a few drops of CHCl<sub>3</sub>.

(b) *Ammonium sulfate standard soln.*—Dilute 200 ml of (a) to 1000 ml. 1 ml = 0.1 mg of N. Preserve with CHCl<sub>3</sub>.

(c) *Nessler soln (Folin\*).*—Transfer 37.5 g of KI and 27.5 g of I to 250 ml flask, and add 25.0 ml of H<sub>2</sub>O and 35–40 g of Hg. Shake flask continuously and vigorously 7–15 min., or until nearly all dissolved I has disappeared. (The soln becomes hot.) When the red I soln has begun to pale visibly, though still red, cool in running H<sub>2</sub>O, and continue shaking until reddish color of I has been replaced by greenish color of

\* "Laboratory Manual of Biological Chemistry," New York, 4th ed., p. 293 (1926). The Nessler reagent prepared by usual procedure is, however, equally satisfactory.

double iodide. (Whole operation should not take more than 15 min.) Separate soln from surplus Hg by decantation and washing with liberal quantities of  $H_2O$ . Dilute soln and washings to 500 ml. (If cooling was begun in time, the resulting concentrated soln of double iodide is clear enough for immediate dilution with 10% NaOH soln and  $H_2O$ , and the finished Nessler reagent can be used at once.) Place 700 ml of 10% NaOH soln in liter flask, add 150 ml of the clear concentrated double iodide soln, mix, and dilute to 1 liter with  $H_2O$ . Allow to settle if turbidity develops.

(d) *Permutit* (Folin\*).—Pass thru sieves and reject material smaller than 80-mesh and larger than 60-mesh. Wash copiously with  $H_2O$  by decantation until the whole settles rapidly and contributes no more dust or turbidity to the  $H_2O$ . Dry in current of air in thin layer without heating. For recovery of permutit after use, see Folin's manual.\*

## 12.59

### DETERMINATION

Transfer an accurately weighed 0.5 g sample of dry finely powdered tobacco to 300 ml Kjeldahl flask; add 25–30 ml of  $H_2O$ , a small piece of paraffin, a few angular quartz pebbles, and 2–2.5 g of light MgO. Prepare stopper to fit Kjeldahl flask with a piece of 9 mm outside diameter glass tubing bent around thru  $180^\circ$ , the short limb of bend inserted thru stopper and the longer limb reaching to level of desk as flask is held in clamp over microburner. (Or, if more convenient, cut longer limb and join again by short length of rubber tubing at point ca 15 cm from lower end.) Connect distillation tube so prepared to flask and dip lower end into a short, wide test tube (50 ml centrifuge tube) that contains 5 ml of 0.1 N HCl and a few drops of methyl red indicator, 2.19(i). Heat contents of flask with micro burner at such a rate that steam begins to rise from receiver in ca 3 min. Make no effort to cool distillation tube or receiver. Distil 5 min., counting time from point at which distillate first runs down tube. Remove tube and wash end into receiver with a few drops of  $H_2O$ . Cool distillate and dilute to 50 ml. Charge several 100 ml volumetric flasks with 2.5–3.0 g of the washed and dried permutit and wash each several times by decantation with  $H_2O$ . Transfer to 3 of the flasks 3 ml of 0.1 N HCl and portions of the standard  $(NH_4)_2SO_4$  soln, 12.58(b), containing 0.3, 0.5, and 1.0 mg of N, respectively. Add sufficient  $H_2O$  to each flask to make total volume of 25 ml. Transfer 25 ml aliquot of distillate from each determination to flask containing permutit. Shake all flasks 5 min. with a gentle rotatory motion and lay them on their sides on suitable support 1 min. Decant fluid from each flask and wash permutit by decantation 3 times successively with 10–30 ml of  $H_2O$ , allowing soln to settle 1 min. before each decantation. Rinse permutit to bottom of each flask with 5 ml of  $H_2O$ , add 1 ml of 10% NaOH soln, and rotate 3 min. Add 65 ml of  $H_2O$ , rotate, and add 10 ml of the Nessler reagent. Dilute to mark, mix, and in colorimeter compare color of soln derived from each determination with known standard that most nearly matches it. (Color is stable several hours.) Report  $NH_3$  nitrogen as percentage of sample of tobacco used.

### FREE NICOTINE IN TOBACCO (24)—TENTATIVE

## 12.60

### DETERMINATION

Mix ca 2.5 g of dry powdered tobacco with 50 ml of  $H_2O$ , stir 5–10 min., and allow to settle. Decant off some of supernatant liquid and determine its pH value with accuracy of 0.1 unit. Construct a curve by plotting data in following table on conveniently large scale. Read percentage of free nicotine from this curve at a point corresponding to pH found and report as percentage of total nicotine in free form.

12.61	FREE NICOTINE per cent	pH	FREE NICOTINE per cent	pH
	1	6.11	50	8.11
	2	6.42	55	8.20
	5	6.86	60	8.29
	10	7.15	65	8.37
	15	7.36	70	8.48
	20	7.51	75	8.59
	25	7.63	80	8.71
	30	7.74	85	8.86
	35	7.85	90	9.06
	40	7.93	95	9.39
	45	8.02		

## NITRATE NITROGEN (25)—TENTATIVE

(Applicable to tobacco and other plant tissues)

## 12.62

## REAGENTS

(a) *Sulfuric acid soln.*—4 *N*. Prepare from C.P. special reagent low in N.(b) *Sulfuric acid soln.*—18 *N*. Prepare from C.P. special reagent low in N.(c) *Reduced iron powder.*—Determine titration value of the  $\text{NH}_3$  in the powder by boiling 3.0 g of it with 50 ml of the 4 *N*  $\text{H}_2\text{SO}_4$  5 min., cooling, making alkaline with NaOH, distilling into 0.1 *N* acid, and titrating with 0.1 *N* alkali to methyl red. Divide by 10 to obtain correction to be used with the 0.3 g of powder used in method.(d) *Ammonium sulfate stock soln.*—Dissolve 2.358 g of  $(\text{NH}_4)_2\text{SO}_4$  in  $\text{H}_2\text{O}$  and make up to 1000 ml. 2 ml = 1.0 mg of N. Add no preservative.(e) *Ammonium sulfate standard soln.*—Dilute 200 ml of (d) to 1000 ml. 1 ml = 0.1 mg of N.(f) *Diphenylamine soln.*—Suspend 0.5 g of diphenylamine in 20 ml of  $\text{H}_2\text{O}$  and add  $\text{H}_2\text{SO}_4$  to make 100 ml. Cool, and preserve in dark bottle.

## 12.63

## DETERMINATION

Ascertain quantity of the 4*N*  $\text{H}_2\text{SO}_4$  required to bring a 2 g sample of the dried and powdered tissue to ca pH 1.0 as follows: Weigh out 0.5 g, stir in small beaker with 1 ml of the 4 *N* acid, add enough  $\text{H}_2\text{O}$  to make a thin paste that can be transferred to electrode vessel, and determine pH of mixture. Make suitable changes in quantity of acid added to a second 0.5 g sample, as suggested by result of first test, and repeat determination. Continue until quantity required to give a reaction in range pH 0.7–0.9 has been found. Multiply this quantity by 4 to obtain amount required by the 2 g sample used for nitrate determination.

Weigh duplicate 2.00 g samples of the powder, mix each in beaker with required quantity of the 4 *N*  $\text{H}_2\text{SO}_4$  until a uniform stiff paste is obtained; add 3.5 g of pure asbestos fiber to each and incorporate thoroly. Transfer mixtures to 26×60 mm paper extraction thimbles by means of glass funnel ca 11 cm long, the upper part of which is a cylinder 4.5 cm in diam., the lower a cylinder 2 cm in diam. Make the transfer as follows: Support thimble in wire cage hung in mouth of 400 ml conical extraction flask and clamp funnel in position over it so that smaller end extends ca 1 cm into thimble. Push most of asbestos mixture into thimble with glass rod; brush off beaker, funnel, and rod; and wipe off all particles with small piece of surgical cotton. Finally rinse glassware and brush into thimble with alcohol-free ether. Remove funnel and plug end of thimble with the cotton used to wipe apparatus. Place thimble in the siphon tube of ether extraction apparatus (type designed for rubber analysis), thrust short slim glass rod between thimble and glass to hold paper away from glass wall at one side, and suspend siphon tube close under

the metal coil condenser of apparatus by means of fine galvanized iron wire. Place 150–200 ml of alcohol-free ether in conical flask. Cut gasket from soft cardboard to fit recess in plate of metal condenser and set condenser, with attached siphon tube, on extraction flask and hold firmly in position by means of 3 spring paper clips.

Place extraction flasks on electric hot plate, add few angular quartz pebbles, and allow extraction to proceed at least 8 hours at siphoning rate of ca 40 times/hour. (If rate is less, correspondingly longer time must be allowed.) To test for completeness of removal of the  $\text{HNO}_3$ , prepare concentrated  $\text{H}_2\text{O}$  extract of residue in thimble and overlay 5 ml of the diphenylamine reagent in test tube with a few ml of this extract. (Appearance of blue layer at junction of the two solns indicates that extraction of the  $\text{HNO}_3$  by the ether has been incomplete.)

Treat each ether extract with 25 ml of  $\text{H}_2\text{O}$ , add 2 drops of phenolphthalein, and make faintly alkaline with 0.5 *N* NaOH with continual agitation. Immerse flask in water bath and evaporate off ether very slowly to avoid frothing; make aqueous soln to 100 ml and transfer aliquot (10 ml or more, depending on nitrate content of tissue) to 300 ml Kjeldahl flask. Add 2.5 ml of the 18 *N*  $\text{H}_2\text{SO}_4$  and 0.3 g of the reduced Fe powder. Boil gently 5 min., cool, add 20 ml of  $\text{H}_2\text{O}$  and 10 ml of  $\text{NH}_3$ -free concentrated NaOH soln. Immediately fit flask with distillation tube and distil as directed under 12.59, into 3 ml of 0.1 *N* HCl contained in test tube. Transfer distillate to 100 ml flask, dilute to ca 60 ml with  $\text{NH}_3$ -free  $\text{H}_2\text{O}$ , add 10 ml of the Nessler soln, agitate, and make to volume. Prepare standard  $\text{NH}_3$  solns by pipetting 3–15 ml of the  $(\text{NH}_4)_2\text{SO}_4$  standard soln into 100 ml flasks (0.3–1.5 mg  $\text{NH}_3$  nitrogen), diluting, adding 10 ml of the Nessler soln, agitating, and making to volume. Read color of soln derived from analysis in colorimeter against nearest standard. Calculate quantity of N in aliquot used for reduction, subtract blank for the  $\text{NH}_3$  nitrogen found in the 0.3 g of Fe powder used, and calculate the nitrate N in the 2.00 g sample taken. Report as percentage of the dry tissue.

If the nitrate content of the tissue is 0.1% or less, it is desirable to carry out a blank determination on the alkaline soln of the ether extract. To do this proceed as follows:

Transfer aliquot of the alkaline soln equal to that used for the determination to 300 ml Kjeldahl flask, add 2.5 ml of the 18 *N*  $\text{H}_2\text{SO}_4$ , boil gently 5 min., cool, add 20 ml of  $\text{H}_2\text{O}$  and 10 ml of NaOH soln, 2.22(g), and distil as directed previously. Transfer distillate to 25 ml volumetric flask, dilute to 15 ml, add 2.5 ml of the Nessler soln, agitate, and make to volume. Compare with  $\text{NH}_3$  standards of 0.05–0.10 mg. Deduct quantity of  $\text{NH}_3$  nitrogen found from quantity found after reduction with the Fe powder, correct result for blank due to Fe powder, and calculate nitrate N as before.

To determine nitrate content of extracts from plant tissue proceed as follows:

Transfer to evaporating dish aliquot of extract ca equivalent to 2 g of dry tissue, make neutral to Congo red if necessary, and evaporate to a sirup (must not be evaporated to dryness). Cool, and add the quantity of the 4 *N*  $\text{H}_2\text{SO}_4$  found by separate experiment to be required to produce reaction in range pH 0.7–0.9; add 3.5 g of asbestos, and mix thoroly. If mixture is too moist to be transferred to extraction thimble, dry it in vacuum desiccator until this can be done. Proceed with extraction as already described.

#### LIGNIN (56)—TENTATIVE

##### 12.64

##### PREPARATION OF SAMPLE

Grind plant material in mill to pass thru 80-mesh sieve, and dry at 105°. Extract weighed sample (5–10 g) 30 hours in Soxhlet apparatus with alcohol-benzene soln

(32 parts by weight of alcohol and 68 parts by weight of benzene). Dry material in oven to free it from the alcohol-benzene soln and place in flask of suitable size. Add  $\text{H}_2\text{O}$  in proportion of 150 ml to 1 g of sample, and boil mixture under reflux condenser 3 hours. Filter mixture while still hot, preferably thru weighed sintered-glass crucible, and transfer extracted material to flask. Add 1%  $\text{HCl}$  soln in proportion of 150 ml of acid soln to 1 g of plant material, and boil under reflux condenser 3 hours. Filter mixture while still hot thru the sintered-glass crucible used in previous operation, wash with  $\text{H}_2\text{O}$  until free of acid, dry at  $105^\circ$ , and weigh. Calculate percentage total loss due to successive extraction with the alcohol-benzene soln, hot  $\text{H}_2\text{O}$ , and the 1%

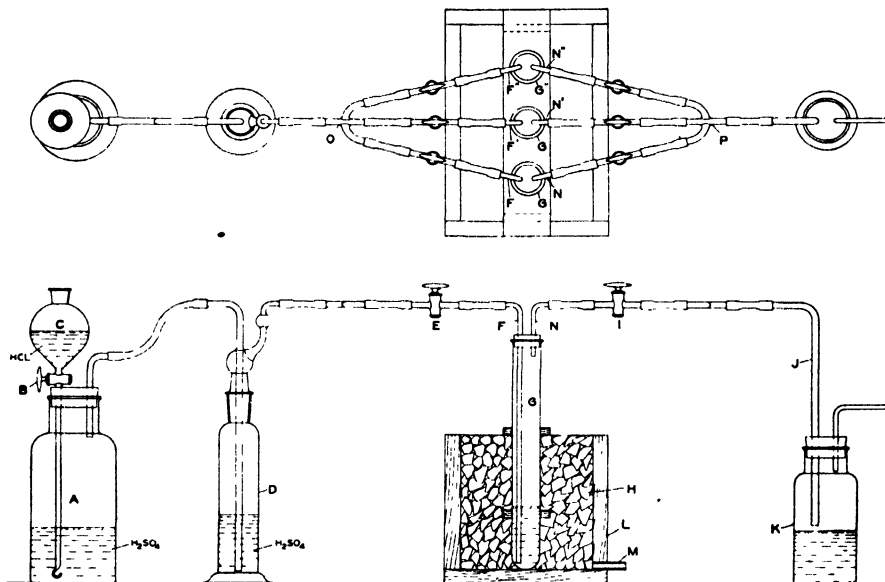


FIG. 18.—APPARATUS FOR DETERMINATION OF LIGNIN

$\text{HCl}$ . (In substances not especially rich in carbohydrates and proteins, extraction with hot  $\text{H}_2\text{O}$  may be omitted.)

## 12.65

### APPARATUS

The apparatus required is illustrated in Fig. 18. It consists of a bottle (A) having a capacity of 1500 ml and containing ca 500 ml of  $\text{H}_2\text{SO}_4$ . Attached to A by means of a 2-holed rubber stopper is a 250 ml dropping funnel (C), having the lower end of its stem bent as illustrated, containing  $\text{HCl}$ . By means of stopcock B, allow the  $\text{HCl}$  to flow into the  $\text{H}_2\text{SO}_4$ , and dry the  $\text{HCl}$  gas thus generated by passage thru the  $\text{H}_2\text{SO}_4$  in D. The lower end of the stem of C must be close to the bottom of A, as shown in drawing. Place weighed sample and the fuming  $\text{HCl}$  in Pyrex test tube 300 mm long and 38 mm in diam. (G). By means of the device O, connect G in parallel to two other tubes ( $G'$  and  $G''$ , see top view), having same dimensions as G, and provide G,  $G'$ , and  $G''$  with 2-holed rubber stoppers. Thru one hole pass glass tube having right-angled bend nearly to bottom of large test tube (F,  $F'$ , and  $F''$ ). Thru other hole insert another tube having right-angled bend, which extends ca 10 mm into the large test tube. K is bottle containing  $\text{H}_2\text{O}$  for absorption

of excess HCl gas that passes thru device P and tube J. Regulate flow of HCl gas thru the three large test tubes by means of the stopcocks shown in top view. Place G, G', and G'' in the wooden box L, provided with supports for the tubes and also with a drain (M) for removal of H<sub>2</sub>O. Surround large test tubes G, G', and G'' in L with crushed ice (H).

## 12.66

## REAGENT

*Fuming hydrochloric acid.*—Density 1.212–1.223 at 15°. To 500 g of NaCl contained in a liter Pyrex distilling flask provided with ground-glass stopper, add cold soln of 250 ml of H<sub>2</sub>O in 450 ml of H<sub>2</sub>SO<sub>4</sub>. Connect side tube of distilling flask to glass tube, which passes thru a H<sub>2</sub>SO<sub>4</sub> wash bottle. Connect outlet tube of the H<sub>2</sub>SO<sub>4</sub> wash bottle to another glass tube, which is immersed in flask containing 3 liters of HCl. Surround flask containing the HCl with crushed ice. Heat distilling flask with small flame and pass the HCl gas into the acid soln until it attains sp. gr. of 1.212–1.223 at 15°. Keep reagent in refrigerator maintained at 0° or below. (If only a few determinations are to be made, a correspondingly smaller quantity should be prepared.)

## 12.67

## DETERMINATION

Weigh out three 1 g samples of the extracted and dried material in weighing bottle and place in the three large test tubes, G, G', and G''. Add 20 ml of the reagent to each tube, taking care to wash down with this acid any particles clinging to sides. When all material is wetted with the reagent, add another portion (30 ml). Add ca 3 drops of capryl alcohol to reduce foaming to minimum during subsequent passage of the HCl gas thru reaction mixture. Place the three large test tubes, G, G', and G'', in the wooden box (L) and surround with crushed ice. Lubricate tubes F, F', and F'' with a drop of glycerol so that they move easily thru holes in rubber stoppers. Lead the dry HCl gas from generator into reaction mixtures thru tubes F, F', and F'' (F' and F'' are shown in top view), which reach nearly to bottom of tubes G, G', and G''. Regulate flow of gas thru reaction mixtures in G, G', and G'' by means of stopcocks shown in top view, continuing passage of the gas 2 hours. (At first a rather slow stream of gas passes in, but during last 15 min. flow is fairly rapid.) At end of reaction period discontinue flow of gas, and disconnect long tubes F, F', and F'' and the outlet tubes of three test tubes G, G', and G'' from O and P. (Tubes F, F', and F'' are pulled up just above surface of reaction mixture and are closed by means of short pieces of rubber tubing having one end plugged with short piece of glass rod.) Similarly close off outlet tube N and the outlet tubes of G' and G''. Place the tubes containing reaction mixture in cold room or icebox (temp. +8°–+10°) and allow to remain 24 hours. Transfer contents of tubes G, G', and G'' to liter Erlenmeyer flasks, taking care to remove any material adhering either on inside or outside of tubes F, F', and F''. Dilute reaction mixtures with H<sub>2</sub>O to volume of 500 ml. Connect flasks to reflux condensers and boil 1 hour. Prepare three Gooch crucibles in usual manner, dry at 105°, and weigh. Ignite one of weighed crucibles, A, on a Bunsen burner, cool in a desiccator, and reweigh. Allow contents of flasks to cool to room temp. and filter thru weighed Gooch crucibles. Wash precipitates collected in Gooch crucibles with hot H<sub>2</sub>O, dry at 105°, and weigh in weighing bottle. Ignite the crude lignin in crucible A over Bunsen flame and determine weight of ash. Place one of the other two Gooch crucibles in Kjeldahl flask provided with wide neck, and determine percentage of N in the crude lignin as directed in 2.26. If it is desired to determine percentage of methoxyl in the lignin, collect precipitate from one of the flasks in a dried (105°) sintered glass crucible and proceed as directed under 41.2. Compute weight of lignin in sample as follows:



Weight of lignin = weight of crude lignin — weight of ash — weight of crude protein ( $N \times 6.25$ ). Calculate percentage of lignin in the original dry unextracted material.

#### CHLOROPHYLL—TENTATIVE

##### *Photoelectric Colorimetric Method for Total Chlorophyll Only (27)*

#### 12.68

##### APPARATUS

(a) *Filter paper*.—Good grade of quantitative paper to fit Büchner funnels if used.

(b) *Flasks*.—Suction flasks of 500 ml capacity and volumetric flasks of 100–500 ml capacity.

(c) *Funnels*.—Small Büchner funnels or sintered-glass funnels of medium porosity.

(d) *Mortar and pestle*.—A deep glass mortar ca 4" in diam., with a well-defined lip, is recommended.

(e) *Photoelectric colorimeter*.—Calibrate for chlorophyll by means of a plant extract as directed in 12.70, using light filters that give maximum light transmission near 6600 Å. (A combination of Corning H. R. light filters Nos. 243 and 396 is suitable for this purpose.)

(f) *Wash bottles*.—The type fitted with a rubber bulb, which permits operation with one hand, is recommended.

(g) *Waring Blendor or similar machine*.—Vessels similar to No. 3, shown in *J. Assoc. Official Agr. Chem.*, 25, 583 (1942), possess advantages over the original Blendor container.

(h) *Wiley mill or similar grinding machine*.—If dried samples are to be analyzed.

(i) *Hand shears or scissors and rubber policeman*.

#### 12.69

##### REAGENTS

(a) *Acetone*.—Undiluted acetone and 85% soln by volume. Commercial acetone of technical grade is satisfactory.

(b) *Calcium or sodium carbonate*.

(c) *Quartz sand*.—Acid-washed and dried.

#### 12.70

##### DETERMINATION

Select field material carefully to insure a representative sample. Remove a representative portion from field sample, and if fresh material, cut finely with hand shears and mix as thoroly as possible. Grind dried material in mill and mix thoroly. Weigh 1–5 g into the mortar and add small quantity (ca 0.1 g) of  $\text{CaCO}_3$  or  $\text{Na}_2\text{CO}_3$ . Macerate tissue with pestle, add the quartz sand, and grind for a short time; then add the 85% acetone soln, a little at a time, and continue grinding until tissue is finely ground. Transfer mixture to funnel, draw off soln with suction, and wash residue with the 85% acetone soln. Return residue to mortar with more acetone soln and grind again. Filter, and wash as before. Repeat this procedure until tissue is devoid of green color and washings are colorless. (It is advisable to grind the residue at least once with undiluted acetone and then to add sufficient  $\text{H}_2\text{O}$  at the end to bring it to 85% soln. A Waring Blendor or similar machine may be used for macerating and extracting the tissue instead of the mortar (see 12.73), but each investigator should satisfy himself that complete extraction of the tissue is accomplished by the device used.) When extraction is complete, transfer filtered extract to volumetric flask of appropriate size and make to volume.

Measure percentage of light transmitted by the soln with photoelectric colorimeter that has been calibrated for chlorophyll by use of a plant extract as described below.

Read amount of chlorophyll present from curve relating light transmission and concentration. Express chlorophyll values as mg/g of tissue, or in other convenient manner.

Calibrate the photoelectric colorimeter as follows: Extract a sample of fresh, green leaf material with the 85% acetone soln, filter, wash residue, and make extract to volume as directed above. Make series of dilutions of extract and measure the % of light transmitted by original and by each of diluted solns with the instrument in the same manner as when a chlorophyll preparation is being used as the calibration standard. Transfer an aliquot of the original extract to ether and evaluate total chlorophyll by the spectrophotometric method as directed in 12.73 (b) and (c). From the value thus obtained calculate chlorophyll content of original extract and that of each of diluted solns, and construct curve relating concentration of chlorophyll and % of light transmitted, or the  $\log_{10} I_0/I$  values, as usual.

*Spectrophotometric Method for Total Chlorophyll and the  
a and b Components (28, 29)*

## 12.71

## APPARATUS

The apparatus listed under 12.63 with the exception of the photoelectric colorimeter and the Wiley mill, plus the following:

(a) *Bottles*.—Small reagent bottles of ca 60 ml capacity, with either glass or cork stoppers, are convenient receptacles in which to bring the ether solns to the proper dilution.

(b) *Pipets*.—Volumetric pipets of various capacities are required for making necessary dilutions, also straight dropping pipets (medicine droppers).

(c) *Scrubbing tubes for washing ether solns*.—Open tube of ca 20 mm diam., to one end of which is sealed tube of smaller diameter drawn to fine jet at lower end.

(d) *Separators*.—250–500 ml capacity.

(e) *Double-deck separator support*.

(f) *Spectrophotometer*.—Must be capable of isolating a spectral region of ca 30 Å near 6600 Å with negligible stray radiation. Tubulated cells with tightly fitting glass stoppers are recommended for work with ether.

## 12.72

## REAGENTS

Those listed under 12.69 plus the following:

(a) *Ether*.—Commercial ether designated as purified for fat extraction is satisfactory without further purification.

(b) *Sodium sulfate*.—Anhydrous.

(c) *Trisodium phosphate*.—Wash glassware with strong soln of this salt in order to remove traces of acid that might tend to decompose chlorophyll.

## 12.73

## DETERMINATION

(a) *Extraction of chlorophyll from tissue*.—Select and prepare sample as directed in 12.70. Disintegrate a weighed portion (2–10 g, depending upon chlorophyll content) of fresh plant tissue in Waring Blendor cup, which contains small quantity (ca 0.1 g) of  $\text{CaCO}_3$ , or by use of a mortar as described in 12.70. After the tissue is thoroly disintegrated, filter extract thru Büchner funnel fitted with quantitative filter paper. Wash residue with the 85% acetone soln, and if necessary use a little ether to remove last traces of pigment. If extraction is incomplete, return residue and filter paper to Blendor container with more acetone soln and repeat extraction. Filter, and wash as directed previously into flask containing first filtrate. Transfer filtrate to volumetric flask of appropriate size and make to volume with the

85% acetone. Pipet an aliquot of 25–50 ml into a separator containing ca 50 ml of ether. Add H<sub>2</sub>O carefully until it is apparent that all the fat-soluble pigments have entered the ether layer. Draw off and discard water layer. Place separator containing ether soln in upper rack of support. Add ca 100 ml of H<sub>2</sub>O to second separator and place it in rack below the first. Set scrubbing tube in place and allow ether soln to run thru it to bottom of lower separator and rise in small droplets thru the H<sub>2</sub>O. When all soln has left upper separator, rinse it and scrubbing tube with a little ether added from medicine dropper. Place scrubbing tube in upper separator and exchange its place in the support with the separator now containing the ether soln. Draw off and discard the H<sub>2</sub>O in upper separator, add a similar portion of fresh H<sub>2</sub>O to lower separator, and repeat washing process. Continue washing ether soln until all acetone is removed (5–10 washings). Then transfer ether soln to 100 ml volumetric flask, make to volume, and mix.

(b) *Spectrophotometric measurements.*—Add ca teaspoonful of anhydrous Na<sub>2</sub>SO<sub>4</sub> to 60 ml reagent bottle, and fill it with the ether soln of the pigment. When this soln is optically clear, pipet an aliquot into another dry bottle and dilute it with sufficient dry ether to cause the  $\log_{10} I_0/I$  value to fall between 0.2 and 0.8 at wave length to be used. The most favorable value is near 0.6 at 6600 Å, since such a soln will yield a satisfactory value at 6425 Å. Fill two clean glass-stoppered absorption cells with dry ether by use of pipet, and polish outside surfaces of each, first with cotton wet with alcohol and then with dry cotton. Place cells in instrument, and determine whether each gives the same galvanometer deflection. If not, clean again or select cells that do, and do this daily. Empty one cell, fill it with the dried ether soln, and place in instrument. Adjust entrance and exit slits until spectral region isolated is 30–40 Å at 6600 Å. Determine whether instrument is in proper adjustment for wave length by taking readings through the solvent and the soln at intervals of 10 Å from 6580–6650 Å. Calculate the  $\log_{10} I_0/I$  value for each wave length at which readings were taken. The highest value should occur at 6600 Å; if it does not, adjust machine until it does or make the 6600 Å readings at the wave length setting that gave the highest value. In the case of a grating instrument apply the same correction at 6425 Å; however, with a prism instrument the correction at 6425 Å must be obtained from a wave length calibration curve for the particular instrument in use. Calibrate instrument for wave length in this way often enough to insure that it remains in proper adjustment. Take  $I_0$  and  $I$  readings at 6600 and 6425 Å (or the corrected settings) for each unknown soln.

(c) *Calculation of chlorophyll concentration.*—Calculate the  $\log_{10} I_0/I$  values for each of the readings made, substitute them in the following simplified equations, and solve for total chlorophyll and each of the  $a$  and  $b$  components as follows:

$$(1) \text{ Total chlorophyll (mg/1)} = 7.12 \log_{10} \frac{I_0}{I} \text{ (at 6600 Å)} + 16.8 \log_{10} \frac{I_0}{I} \text{ (at 6425 Å)}.$$

$$(2) \text{ Chlorophyll } a \text{ (mg/1)} = 9.93 \log_{10} \frac{I_0}{I} \text{ (at 6600 Å)} - 0.777 \log_{10} \frac{I_0}{I} \text{ (at 6425 Å)}.$$

$$(3) \text{ Chlorophyll } b \text{ (mg/1)} = 17.6 \log_{10} \frac{I_0}{I} \text{ (at 6425 Å)} - 2.81 \log_{10} \frac{I_0}{I} \text{ (at 6600 Å)}.$$

#### SUPPLEMENTARY INFORMATION

The factors involved in the spectrophotometric analysis of the chlorophyll system have been discussed in detail by Comar and Zscheile (29). These authors used Beer's law in the form:

$$c = \frac{\log_{10} \frac{I_0}{I}}{\alpha l},$$

where  $I_0$  is intensity of light transmitted by solvent-filled cell;  $I$  is intensity of light transmitted by soln-filled cell;  $c$  is concentration of chlorophyll (g/liter);  $\alpha$  is specific absorption coefficient, and  $l$  is thickness of soln layer in cm.

Since at a given wave length the observed  $\log_{10} I_0/I$  value of a soln having two components represents the sum of the  $\log_{10} I_0/I$  values of each of the components, the following equation obtains in the case of chlorophylls  $a$  and  $b$  at a given wave length:

$$(4) \quad \left( \log_{10} \frac{I_0}{I} \right)_{\text{observed}} = \left( \log_{10} \frac{I_0}{I} \right)_a + \left( \log_{10} \frac{I_0}{I} \right)_b.$$

If a 1 cm cell is used this equation may be expressed as:

$$(5) \quad \left( \log_{10} \frac{I_0}{I} \right)_{\text{observed}} = \alpha_a c_a + \alpha_b c_b.$$

The concentrations of chlorophylls  $a$  and  $b$  in a given ether soln can now be calculated by the use of equation (5) as follows:

(a) Determine  $\log_{10} I_0/I$  values for the soln at two different wave lengths (6600 and 6425 Å have been found advantageous for this purpose).

(b) Select proper specific absorption coefficients corresponding to the wave lengths used from the table.

(c) Substitute the observed  $\log_{10} I_0/I$  value and the specific absorption coefficient in equation (5) for each of the two wave lengths used as illustrated for 6600 and 6425 Å in equations (6) and (7). Solve these two equations simultaneously for the two unknowns, the concentrations of chlorophylls  $a$  and  $b$ .

$$(6) \quad \log_{10} \frac{I_0}{I} \text{ (at 6600 Å)} = 102c_a + 4.50c_b.$$

$$(7) \quad \log_{10} \frac{I_0}{I} \text{ (at 6425 Å)} = 16.3c_a + 57.5c_b.$$

Equations (1), (2), and (3) were derived in this way.

The criterion for accuracy of the chlorophyll values as determined by spectrophotometric method is the agreement between analytical results as determined from measurements at different wave lengths. It has been demonstrated by Comar and Zscheile (29) that measurements at 6600 and 6425 Å are convenient for routine analysis; however, readings may be made at other wave lengths to check these values. Specific absorption coefficients for chlorophylls  $a$  and  $b$  in ether soln that may be used for this purpose are presented in the following table:

12.74 Absorption constants used in analysis (after Comar and Zscheile (29) )

WAVE LENGTH Å	SPECIFIC ABSORPTION COEFFICIENTS (FOR ETHER SOLNS)	
	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>
6600	102.	4.50
6425	16.3	57.5
6000	9.95	9.95
5810	8.05	8.05
5680	7.11	7.11
6130	15.6	8.05
5890	5.90	10.3

These values may be used for calculations as follows:

(a) Values for total chlorophyll and percentage composition may be calculated from absorption values at 6600 and 6425 Å as described.

(b) Check values for total chlorophyll may be calculated from the absorption values at the intersection points 6000, 5810 and 5680 Å.

(c) Check values for percentage composition may be calculated from the absorption values for each of the points 6130 and 5890 Å in combination with a value of total concentration as obtained from (a) or (b).

#### CAROTENE

(See also 20.55–20.59 and 36.7–36.15)

#### 12.75

##### *Barium Hydroxide Method (30)—Tentative*

(a) *If chlorophyll is determined in the same sample.*—Pipet 50 ml aliquot of the acetone soln, 12.69 or 12.73, into 300 ml Erlenmeyer flask containing 1 g of finely divided  $\text{Ba}(\text{OH})_2$ , or proportional amount of  $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ , free from carbonate. Shake until thoroly mixed, wash down sides of flask with 85% acetone, attach flask to condenser, and reflux for 30 min., shaking flask occasionally during refluxing process. Detach flask from condenser and cool. Filter yellow soln from  $\text{Ba}(\text{OH})_2$ -chlorophyll mixture thru sintered-glass funnel of medium porosity or thru quantitative filter paper in Büchner funnel, and wash with 85% acetone soln until washings are colorless. Pour soln into 500 ml separator containing 50 ml of petroleum benzine. Rinse suction flask with three 10 ml portions of 85% acetone and add them to separator. Swirl contents of separator gently to collect as much yellow pigment in the petroleum benzine as possible. Permit layers to separate and draw off acetone layer into second separator. Extract this soln three times with 10–15 ml portions of petroleum benzine and add them to first separator. Discard acetone soln. Wash petroleum benzine extract twice with 20 ml portions of  $\text{H}_2\text{O}$  and discard washings. Extract the petroleum benzine soln with 30 ml portions of 90% methanol saturated with petroleum benzine until alcoholic extract is colorless. Extract the methanol washings once with 10–15 ml of petroleum benzine. Separate and add extract to carotene soln in separator. Wash 2 or 3 times with  $\text{H}_2\text{O}$  to remove traces of alcohol. Dry stem of separator and filter petroleum benzine soln into 100 ml volumetric flask thru fluted filter paper containing small quantity of anhydrous  $\text{Na}_2\text{SO}_4$  as drying agent. Wash paper free of yellow color with petroleum benzine, make to volume, and mix.

Evaluate concentration of carotene in this soln by use of (1) photoelectric colorimeter or (2) spectrophotometer:

(1) Measure per cent of light transmitted thru a portion of the soln by means of photoelectric colorimeter equipped with light filter with appropriate spectral characteristics (Corning No. 554 H. R. Lantern Blue polished light filter of standard thickness is suitable). Read concentration of carotene from previously constructed transmission-concentration curve. To test for traces of chlorophyll in soln take reading thru a filter combination that transmits maximum light intensity near 6600 Å (12.68 (e)). If reading thru soln is same as thru solvent no chlorophyll is present. However, if reading thru soln is less than thru solvent chlorophyll is present and reading for

carotene should be corrected by use of following formula:  $Y = \frac{T \times 100}{C}$ , where  $Y =$

true % transmission of carotene;  $T =$  total % transmission of carotene and chlorophyll thru blue filter; and  $C =$  % transmission of chlorophyll concentration thru blue filter in absence of carotene. (To obtain this it is necessary to have transmission-

concentration curve for chlorophyll for each of red and blue filter combinations. Chlorophyll in soln is evaluated by using reading thru red filter with corresponding curve. C, or transmission this amount of chlorophyll would show thru blue filter, is then read from other curve and used in above formula). Use corrected transmission value to obtain concentration of carotene from carotene curve. For (2) see 36.11.

(b) *If chlorophyll is not to be determined on sample.*—Proceed with extraction of pigments from fresh tissue as directed under 12.70 as far as "Transfer mixture to funnel . . .". Instead transfer mixture to 200–300 ml Erlenmeyer flask containing ca 1 g of  $\text{Ba}(\text{OH})_2$ , using 85% acetone soln to effect transfer. Add more of this soln until volume is ca 75 ml. Weigh finely ground dry samples directly into Erlenmeyer flask containing 1 g of  $\text{Ba}(\text{OH})_2$  and 75 ml of 85% acetone soln. In either case agitate flask until contents are thoroly mixed and wash down sides of flask with more acetone soln. Attach flask to condenser and reflux 30 min. Remove flask, cool, and proceed as directed in (a) from point where directed to "Filter yellow soln from  $\text{Ba}(\text{OH})_2$ -chlorophyll mixture thru sintered-glass funnel . . ."

**12.76**      *Chromatographic Isolation of Carotene by use of Dicalcium Phosphate (31)—Tentative*

Grind dried materials fine, run silage thru food chopper, and cut fresh material's as fine as possible with scissors. Use samples of 2–4 g, depending upon carotene content. Grind silage and fresh material in mortar with quartz sand and small quantity of aldehyde-free alcohol until fine. Transfer mixture to 250 ml Erlenmeyer flask with enough alcohol to bring volume to 50 ml. Weigh samples of dried material directly into flask and add alcohol. Reflux mixture 30 min. on water bath, cool, and decant extract into 500 ml separator. Add 25 ml of alcohol to residue, reflux 30 min., and again decant extract into separator. Extract residue several times with portions of mixture of ca 25 ml of alcohol and 50 ml of petroleum benzine and decant each time into separator. Break soln into layers by adding sufficient  $\text{H}_2\text{O}$  to make concentration of alcohol ca 80%.

Draw off aqueous-alcohol layer and extract it 3 times with 30 ml portions of petroleum benzine, which are added to first petroleum benzine extract. Discard aqueous-alcohol mixture.

Remove all alcohol from petroleum benzine soln by washing 6–7 times with 100 ml portions of  $\text{H}_2\text{O}$  and concentrating to ca 30 ml. (All alcohol must be removed from petroleum benzine soln, otherwise pigments other than carotene will not be adsorbed on the chromatographic column.)

Make tube for holding column of dicalcium phosphate from test tubes ca 2.2 cm in diameter and 19 cm long by blowing out bottom and sealing on stem ca 12 cm long and 7 mm diameter. Insert stem thru one hole of 2-holed rubber stopper that will fit a 250 ml Erlenmeyer flask and insert a glass tube bent at right angle into other hole. Place stopper in Erlenmeyer flask clamped to ring stand for support and attach right angle bend to suction with rubber tubing. Plug opening in bottom of tube with wad of cotton and fill ca half full with  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ , which is packed lightly by application of suction and light tamping with small plunger made of glass rod and cork stopper of proper size. Place small amount of anhydrous  $\text{Na}_2\text{SO}_4$  on top of column and cover this with layer of cotton. Pour 30 ml of petroleum benzine into tube and draw into column with gentle suction. Before all petroleum benzine enters column add petroleum benzine soln of pigments concentrated to 20–30 ml, and follow this with 60 ml of petroleum benzine. Concentrate filtrate containing carotene to convenient volume if necessary, transfer to volumetric flask, bring to volume, and mix. Evaluate concentration of carotene as directed in 12.75(a), (1) or (2).

*Alternative extraction procedure (32).*—Extraction of carotene from samples may be accomplished with a mixture of alcohol and petroleum benzine in a Waring Blendor instead of refluxing with alcohol as described above. Prepare samples as directed and weigh 1–4 g portions of fresh material into Blendor vessel containing a mixture of 4 volumes of alcohol and 3 volumes of petroleum benzine. Weigh sample of dried material onto filter paper in small Büchner funnel and add ca 25 ml of  $H_2O$  at 60–70°. Draw off excess  $H_2O$  with light suction and add paper and sample to Blendor vessel. Place lid on vessel and run Blendor for ca 5 min. or such time as is necessary to macerate tissue. If mixture does not foam sufficiently to prevent splashing during maceration add alternately small portions of alcohol and petroleum benzine until foaming is achieved. Permit residue to settle and decant supernatant liquid into 500 ml separator. Add sufficient  $H_2O$  to bring concentration of alcohol to ca 80%, and after layers separate draw off alcohol layer into another separator. Extract residue in Blendor vessel with 30 ml of petroleum benzine, allow layers to separate, draw off alcohol layer, and add petroleum benzine layer to original extract. Extract residue and alcohol soln twice more with 30 ml portions of petroleum benzine. Continue as directed above, beginning “Remove all alcohol from petroleum benzine soln. . . .”

## 12.77

## SUPPLEMENTARY INFORMATION

The determination of carotene in different kinds of plant tissue may sometimes be facilitated by combining features from several methods into a procedure well suited for a given material. In some instances time can be saved by omitting unnecessary steps and in others use of the right technic may mean the difference between obtaining reliable and unreliable values.

The following suggestions are designed to aid in this direction:

(1) With plant parts devoid of chlorophyll, such as carrot roots, sweet potato roots, peaches, musk melons, squash, etc., it is usually unnecessary to take measures to separate this pigment. Consequently, digestions and extractions may be made without use of alkalis. In fact, in cases where the carbohydrate content is high, e.g. with sweet potatoes, it is advisable to omit the alkali (33). Carotene may be isolated from such tissues easily by macerating them in a mixture of alcohol and petroleum benzine with a Waring Blendor as described above under “Alternative Extraction Procedure,” filtering thru cloth into separator, separating layers, and removing xanthophylls from the petroleum benzine soln with 90% methanol.

(2) Numerous investigators use Skellysolve instead of petroleum benzine.

(3) The  $Ba(OH)_2$  method removes chlorophyll from extracts of fresh green tissue more effectively than it does chlorophyll that has been altered in cooking or dehydration processes. Traces of chlorophyll may be separated from petroleum benzine soln by adding 25–50 ml of ca 0.5 *N* alcoholic KOH soln, shaking vigorously, allowing to stand for short time, and washing out soluble chlorophyll residues with  $H_2O$ .

(4) The  $Ba(OH)_2$  may be added in saturated soln instead of in solid form if desired.

(5) Alcohol may be used for the extractant in the  $Ba(OH)_2$  method instead of acetone if desired.

(6) Emulsions between petroleum benzine and methanol can be broken quickly by running a few drops of alcohol down the side of the separator.

## 12.78

## TOTAL BORON (34)

Place 0.25–0.50 g of plant material (oven-dried and ground) in Pt crucible or porcelain evaporating dish and ignite in muffle furnace at 450° for 8 hours, or until carbon-

free ash is obtained. After cooling, add 5 ml of ca 0.36 N  $H_2SO_4$  and triturate with policeman. After filtering, place 1 ml of the clear filtrate in comparison tube, add 10 ml of the quinalizarin- $H_2SO_4$  soln, 1.28(f), stopper tube, and mix thoroly by whirling gently. Cool to 25° and then make readings in colorimeter as directed in 1.29. Report result as p.p.m. of B.

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### 13. BEVERAGES (NON-ALCOHOLIC) AND CONCENTRATES

13.1

#### PRELIMINARY EXAMINATION

Note and record (a) appearance, whether bright or turbid, or any sediment; (b) color and depth of color; (c) odor, whether fruity, foreign, or artificial; (d) taste, whether tart or sweet, fruity, artificial or foreign, and whether any synthetic substance can be identified by odor or taste.

13.2

SPECIFIC GRAVITY—OFFICIAL.—See 16.4

13.3

ALCOHOL—OFFICIAL.—See 16.6—16.7

13.4

TOTAL SOLIDS—OFFICIAL.—See 34.4 or 34.5

#### SUCROSE

13.5

*By Polarization—Official*

Determine by polarizing before and after inversion as directed under 34.23 or 34.24.

13.6 *By Reducing Sugars Before and After Inversion—Official.—See 34.30*

13.7

#### REDUCING SUGARS—OFFICIAL

Use the value for reducing sugars before inversion, 13.6

13.8

COMMERCIAL GLUCOSE—OFFICIAL.—See 34.32

13.9

#### ASH—OFFICIAL

Proceed as directed under 34.9 or 34.10, using a quantity of sample that contains not more than 10 g of solids.

13.10

#### SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under 34.13, using ash obtained under 13.9.

13.11

#### ALKALINITY OF SOLUBLE ASH—OFFICIAL

Proceed as directed under 34.14, using soluble ash obtained under 13.10.

13.12

#### ALKALINITY OF INSOLUBLE ASH—OFFICIAL

Proceed as directed under 34.15, using insoluble ash obtained under 13.10.

13.13

ANALYSIS OF THE ASH—OFFICIAL.—See Chap. 26

13.14

PRESERVATIVES AND ARTIFICIAL SWEETENERS—OFFICIAL.—See Chap. 32

13.15

COLORING MATTERS—TENTATIVE.—See Chap. 21

13.16

METALS—TENTATIVE.—See Chap. 29

13.17

TOTAL ACIDITY—OFFICIAL.—See 16.62

13.18

POLYBASIC ORGANIC ACIDS—PRELIMINARY PROCEDURE—TENTATIVE

(a) *Alcoholic products.*—Proceed as directed under 16.63.

(b) *Non-alcoholic products.*—Measure out volume of sample that contains not more than 30 g of solid matter and not more than 200 mg of the acid to be determined as calculated from the acidity. Evaporate to 30 ml if necessary, add 3 ml of 1 N H<sub>2</sub>SO<sub>4</sub> (except in determining malic acid), and transfer to 250 ml volumetric flask, using 10 ml of H<sub>2</sub>O and sufficient alcohol to fill flask to mark. Mix, and allow to stand 15 min. Filter thru thin layer of absorbent cotton, protecting liquid against evaporation. Transfer 200 ml of filtrate to centrifuge bottle and proceed as directed below.

**13.19 TARTARIC ACID—TENTATIVE**

Using the material in the centrifuge bottle, proceed as directed under 26.33 or 26.35.

**13.20 CITRIC ACID—TENTATIVE**

Using the material in the centrifuge bottle, proceed as directed under 26.37.

**13.21 MALIC ACID—TENTATIVE**

Using the material in the centrifuge bottle, proceed as directed under 26.40 or 26.43.

**13.22 VOLATILE ACIDS—OFFICIAL.—See 15.23****13.23 ESTERS—OFFICIAL.—See 16.68****ANTHRANILIC ACID ESTER***Colorimetric Method (1)—Official*

(Applicable to samples containing less than 500 mg per liter.)

**13.24 REAGENTS**

- (a) *Dilute hydrochloric acid.*—Dilute 83 ml of HCl to 1 liter with H<sub>2</sub>O.
- (b) *Sodium nitrite soln.*—Dissolve 2 g of NaNO<sub>2</sub> in 100 ml of H<sub>2</sub>O.
- (c) *Hydrazine sulfate soln.*—Dissolve ca 3 g of N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>SO<sub>4</sub> in 100 ml of H<sub>2</sub>O.
- (d) *Sodium-α-naphthol-2-sulfonate soln.*—Dissolve 5 g of the sulfonate in 100 ml of H<sub>2</sub>O.
- (e) *Sodium carbonate soln.*—Dissolve 25 g of Na<sub>2</sub>CO<sub>3</sub> in 75 ml of H<sub>2</sub>O.
- (f) *Standard soln of methyl anthranilate.*—Dissolve 0.25 g of methyl anthranilate in 60 ml of alcohol and dilute with H<sub>2</sub>O to 250 ml.

**13.25 APPARATUS**

- (a) *Steam generator filled with H<sub>2</sub>O.*—Oil can holding 1 gallon will serve purpose.
- (b) *Distillation flask.*—Kjeldahl flask of ca 750 ml capacity, with shortened neck, ca 10" in height over all.
- (c) *Spray tube.*—Glass tube with small perforated bulb at end. Passes thru rubber stopper and reaches to bottom of distillation flask.
- (d) *Connecting bulb.*—Kjeldahl bulb with bent connecting tube.
- (e) *Worm condenser.*—Having water jacket 10–12" long. Outlet tube is extended to reach bottom of receiving flask.
- (f) *Receiving flask.*—500 ml Erlenmeyer flask.

**13.26 DETERMINATION**

Place enough H<sub>2</sub>O in receiving flask to just cover or seal end of extended condenser tube. Place 10–100 ml of sample in distillation flask; add, if necessary, sufficient H<sub>2</sub>O to make volume 100 ml; insert stopper carrying spray tube and connecting bulb; and connect with condenser and receiving flask. Immerse distillation flask in water bath to level of contents, and when sample has attained temp. of the nearly boiling bath connect with steam generator and pass rapid current of steam thru sample until ca 300 ml of distillate has been collected.

Disconnect apparatus and wash out condenser with a little H<sub>2</sub>O. Add to distillate 25 ml of the dilute HCl and 2 ml of the NaNO<sub>2</sub> soln, mix well, and let stand exactly 2 min. Add 6 ml of the N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>SO<sub>4</sub> soln and mix well for a minute, so that liquid comes in contact with all parts of flask that may have been touched by the soln when it contained free HNO<sub>2</sub>. Keep liquid in flask in rapid motion, add quickly

5 ml of the Na- $\alpha$ -naphthol-2-sulfonate soln, and then add immediately 15 ml of the Na<sub>2</sub>CO<sub>3</sub> soln. Dilute the colored soln to 500 ml with H<sub>2</sub>O, mix, and compare color of aliquot with color of a standard, or set of standards, prepared as nearly as possible at same time. Calculate and express results as mg of anthranilic acid ester, as methyl anthranilate, per liter of sample.

*Gravimetric Method (2)—Official*

(Applicable to samples containing 500 mg or more per liter)

**13.27**

**REAGENTS AND APPARATUS**

(a)  $\alpha$ -naphthol soln.—Dissolve 0.2 g of  $\alpha$ -naphthol in 100 ml of 30% (by volume) alcohol.

(b) Sodium bicarbonate soln.—Dissolve 8.4 g of NaHCO<sub>3</sub> in 100 ml of H<sub>2</sub>O.

(See 13.24 for other reagents and see 13.25 for apparatus.)

**13.28**

**DETERMINATION**

Place in the distillation flask a quantity of the sample that contains 50–125 mg of anthranilic acid ester and dilute, if necessary, to 100 ml with H<sub>2</sub>O. Subject sample to steam distillation as directed in 13.26, collecting ca 400 ml of distillate. Have the H<sub>2</sub>O in bath near b.p. when bath is placed under distillation flask, also have the H<sub>2</sub>O in steam generator boiling and make connection immediately.

Wash out condenser with a little H<sub>2</sub>O and dilute distillate to 500 ml. Mix, and to 200 ml aliquot add 5 ml of the dilute HCl and 5 ml of the NaNO<sub>2</sub> soln. Mix well and let stand 1 min. Mix 25 ml of the  $\alpha$ -naphthol soln and 6 ml of the NaHCO<sub>3</sub> soln, pour the diazotized soln into mixture, and let stand 10 min. Fold two Whatman No. 1 or S. & S. No. 595 filter papers, 12.5 cm in diam., and determine difference in their weights by placing one on each pan of balance and counterpoising with added weights. Place the heavier inside the lighter paper, fit into funnel, and moisten. Pour mixture thru this filter and wash precipitate 7 or 8 times, using total of ca 100 ml of H<sub>2</sub>O. Fill filter only to within 1 cm of top. Place funnel carrying filter and washed precipitate in oven, and dry ca 10 min. at 100°. Separate filter papers and dry them ca 1 hour at same temp. Ascertain difference in weights, dry again, weigh again, and repeat this procedure until difference in weights remains constant. From this constant difference in weights subtract original difference in weights of the 2 filter papers and multiply result by 0.4935 to obtain weight of anthranilic acid ester, as methyl anthranilate. Report as grams/liter of sample.

**13.29**

**BENZALDEHYDE (3)—TENTATIVE**

Measure into distilling flask 500 ml of the beverage, 100 ml of flavoring sirup, or 10–25 ml of flavor; add 32 ml of alcohol, and in the case of sirup or flavor, ca 300 ml of H<sub>2</sub>O, and proceed as directed in 16.72.

**13.30**

**GAMMA UNDECALACTONE (4)—TENTATIVE**

Proceed as directed under 16.69, using 500 ml of beverage, 100 ml of flavoring sirup, or 10–50 ml of flavor.

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## 14. MALT BEVERAGES, SIRUPS AND EXTRACTS, AND BREWING MATERIALS\*

### BEER

(Unless otherwise directed express results as g per 100 g)

#### 14.1 PREPARATION OF SAMPLE—OFFICIAL

Remove CO<sub>2</sub> by transferring sample to large flask and shaking, gently at first and then vigorously, or by pouring back and forth between beakers, maintaining temp. of beer at 20–25°. Eliminate foam by passing the CO<sub>2</sub>-free beer thru dry filter paper.

#### 14.2 COLOR—OFFICIAL

Proceed as directed under 14.55. Filter or centrifuge beers showing opacity.

#### 14.3 SPECIFIC GRAVITY—OFFICIAL

Determine sp. gr. at 20/20° (in air) as directed under 16.4.

#### 14.4 APPARENT EXTRACT OR SACCHARIMETRIC INDICATION—OFFICIAL, FIRST ACTION

Ascertain apparent extract corresponding to sp. gr. determined at 20/20° from 44.3, reporting to second decimal place.

#### 14.5 ALCOHOL—OFFICIAL

(a) *By volume.*—See 16.6.

(b) *By weight.*—See 16.7.

#### 14.6 REAL EXTRACT—OFFICIAL

(a) Evaporate 75–100 ml of sample (accurately weighed to 0.1 g) on water bath or asbestos plate, at temp. not exceeding 80°, to ca  $\frac{1}{3}$  of its original volume. Cool, make to original weight with H<sub>2</sub>O, and determine sp. gr. with pycnometer at 20/20°. (If too much H<sub>2</sub>O has been added, sp. gr. will be proportionately too low, and correction must be made.) Ascertain real extract directly from 44.3.

(b) If no anti-foam material was used in the determination of alcohol, 14.5, transfer residue quantitatively with hot H<sub>2</sub>O to 100 ml flask. Cool, and make up to 100 ml at 20°. Determine sp. gr. at 20/20°, 14.3, and ascertain extract direct from 44.3. If 100 ml of beer was taken, make following correction:

$$\text{Extract found} \times \frac{\text{sp. gr. of dealcoholized beer}}{\text{sp. gr. of beer}} = \text{g of extract/100 g of beer.}$$

#### 14.7 EXTRACT OF ORIGINAL WORT—TENTATIVE

Calculate from following formula and report to first decimal place:

$$O = \frac{(A \times 2.0665) + E}{100 + (A \times 1.0665)} \times 100, \text{ in which}$$

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\* Most of the methods in this chapter have been modified and tested by both the American Society of Brewing Chemists and the Association of Official Agricultural Chemists and have been adopted by both associations. See "Methods of Analysis, A.S.B.C.," 4th ed., 1944.

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$O$  = extract of original wort;  $A$  = % alcohol by weight (g/100 g of beer); and  $E$  = real extract, 14.6 (a) or (b).

### 14.2 REAL DEGREE OF FERMENTATION OR REAL ATTENUATION—TENTATIVE

Calculate as follows and report to first decimal place:

$$\frac{\text{orig. ext.} - \text{real ext.}}{\text{orig. ext.}} \times 100.$$

### 14.9 APPARENT DEGREE OF FERMENTATION OR APPARENT ATTENUATION—TENTATIVE

Calculate as follows and report to first decimal place:

$$a = \frac{\text{orig. ext.} - \text{apparent ext.}}{\text{orig. ext.}} \times 100,$$

in which  $a$  = apparent degree of fermentation or apparent attenuation.

### TOTAL ACIDITY—TENTATIVE

#### 14.10

#### *Indicator Titration Method*

Bring 250 ml of  $H_2O$  to boil and continue boiling for 2 min. From fast flowing pipet add 25 ml of beer previously decarbonated by shaking and filtering, 14.1. After emptying pipet, continue heating 60 seconds, regulating intensity of heat source so that soln resumes boiling during final 30 seconds. Remove from source of heat, stir 5 seconds, and cool rapidly to room temp.

Add 0.5 ml of 0.5% phenolphthalein indicator. Against white background, titrate with 0.1  $N$  NaOH. Make frequent color comparisons with sample of equal volume and dilution to which has been added the approximate anticipated quantity of alkali but no indicator. Titrate to first appearance of faint pink color. Read buret. Add 0.2 ml more alkali; color should then be permanent, definite pinkish-red, indicative of over-titration. Take first buret reading as end point.

Observe strictly all details of method. However, 100 ml of  $H_2O$ , 10 ml of beer, and 0.2 ml of indicator may be used in place of quantities specified above. (For beers of dark color, which—even when diluted—may not permit judging of phenolphthalein end point with necessary precision, the alternative method of potentiometric titration to pH 8.2 is recommended.)

Report results: (a) as lactic acid, to nearest 0.01% (1 ml of 0.1  $N$  alkali = 0.0090 g of lactic acid); or, (b) as ml of 1.0  $N$  alkali, to the nearest 0.1, necessary for neutralization of 100 g of beer.

#### 14.11

#### *Potentiometric Titration Method*

Use glass-calomel electrode system. Decarbonate beer completely by shaking, 14.1. Repeat shaking and releasing of pressure at least 12 times at ca 5 min. intervals. Filter thru dry paper. Using 50 ml of undiluted sample (or such quantity as best suits the titration assembly) titrate potentiometrically with 0.1  $N$  NaOH to pH value of 8.2. Add alkali in portions of 1.5 ml up to ca pH 7.6, and in portions of 0.15 ml from there to pH 8.2. Make sure that complete equilibrium and good convergence are attained before reading buret at exactly pH 8.2. Report results as directed under 14.10.

**PRECAUTIONS:** Observe all details of good potentiometric technic, including following: Standardization of potentiometer against fresh 0.05  $M$  potassium acid

phthalate before and after any set of titrations; reading of potentiometer to nearest 0.01 unit; use of flexible shielding around electrode leads and motor cords; grounding of motor and motor cords, preferably to water pipes; avoidance of contact between electrodes and glass beaker; proper speed of stirring to assure quick mixing but to avoid foaming which may temporarily trap some of alkali added; stopping titration at not over pH 8.6 to minimize alkali contamination of glass electrode; frequent checks of the batteries. Follow instructions issued by manufacturer of potentiometer used.

#### H-ION CONCENTRATION (pH)—TENTATIVE

##### 14.12

##### *Electrometric Method*

Decarbonate beer completely by shaking in Erlenmeyer flask or wide-mouth bottle. Repeat shaking and releasing of pressure at least 12 times at ca 5 min. intervals. Filter thru dry paper.

Determine pH of undiluted sample so prepared, being careful to follow instructions issued by manufacturer of potentiometer used. Check the pH meter before and after use against standard buffers. (Freshly prepared 0.05 *M* solns of K acid phthalate serve well for standardization of potentiometers at pH 4.00.) Observe precautions under 14.11. Use glass-calomel electrode systems. Report results to nearest 0.05 pH unit.

##### 14.13

##### *Colorimetric Method*

In absence of potentiometric equipment, follow colorimetric procedure, using bromocresol green as indicator. Make sure of clean pipets and undeteriorated color standards. Report results to nearest 0.1 pH unit.

##### 14.14

##### VOLATILE ACIDS—OFFICIAL

Using 100 ml of beer, proceed as directed under 15.24. Express result as acetic acid, g/100 ml. 1 ml of 0.1 *N* alkali = 0.0060 g of acetic acid.

##### 14.15

##### REDUCING SUGARS—OFFICIAL

Dilute 25 ml of prepared sample, 14.1, measured at 20°, to 100 ml with H<sub>2</sub>O at same temp. Determine reducing sugars in 25 ml of this soln by Munson-Walker method as directed under 34.59. or dilute 50 ml of beer with H<sub>2</sub>O to 100 ml and use the Lane-Eynon method as directed under 34.58. Express result as g of maltose/100 ml of beer. For conversion to percent by weight, divide results by sp. gr. of beer.

##### 14.16

##### DEXTRIN—TENTATIVE

To 25 ml of prepared sample, 14.1, measured at 20° in Erlenmeyer flask, add 15 ml of HCl (sp. gr. 1.125) and dilute to 200 ml. Attach flask to reflux condenser, and keep in boiling water bath 2 hours. Cool, nearly neutralize with NaOH, make up to volume of 250 ml, filter, and determine dextrose as directed under 34.51 or 34.53. From number of g of dextrose/100 ml of beer, subtract 1.053 times quantity of maltose, 14.15, and multiply remainder by 0.9. The result is number of g of dextrin /100 ml of beer.

##### 14.17

##### GLYCEROL—OFFICIAL.—See 15.6

##### 14.18

##### ASH—OFFICIAL

Evaporate to dryness 50 ml of prepared sample, 14.1, measured at 20°, and proceed as directed under 34.9 or 34.10.

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### 14.19

#### PHOSPHORIC ACID—OFFICIAL

To 50 ml of prepared sample, 14.1, measured at 20°, add 20 ml of 2% Ca acetate soln, evaporate to dryness, and ignite at low redness to white ash. Add 10–15 ml of boiling  $\text{HNO}_3$  (1+9) and determine  $\text{P}_2\text{O}_5$  as directed under 2.12.

### 14.20

#### PROTEIN—OFFICIAL

To 25 ml of prepared sample, 14.1, at 20° in Kjeldahl digestion flask, add 2–3 ml of  $\text{H}_2\text{SO}_4$  (1+1), and concentrate to sirupy consistency. Determine N as directed under 2.24, 2.25, or 2.26. Multiply result by 6.25 to calculate percentage of protein.

#### CARBON DIOXIDE—TENTATIVE

##### *Chemical Method*

### 14.21

#### APPARATUS

Apparatus consists of 250 ml tall Drechsel gas-washing bottle connected to beer bottle by small distilling head. Orifice of intake tube in Drechsel gas-washing bottle is constricted to diam. of ca 1 mm at point where gas enters KOH soln. Beer bottle is contained in large beaker.

### 14.22

#### REAGENTS

(a) *Sulfuric acid soln.*—Approximately 2.25 *N*. Standardize as follows: Weigh out 4–6 g of anhydrous  $\text{Na}_2\text{CO}_3$ , dissolve in ca 200 ml of  $\text{H}_2\text{O}$  in 500 ml Erlenmeyer flask, and titrate to phenolphthalein and methyl orange end points. Use difference between these two end points to calculate titer of acid (ca 0.1 g of  $\text{CO}_2$ /ml).

(b) *Potassium hydroxide soln.*—Approximately 5 *N*. Dissolve 280 g of C.P. KOH in 1 liter of  $\text{H}_2\text{O}$ . Avoid excessive exposure to air.

(c) *Special phenolphthalein indicator.*—Dissolve 0.6 g of thymolphthalein and 1.0 g of phenolphthalein in 60 ml of alcohol. Dilute with 40 ml of  $\text{H}_2\text{O}$ .

(d) *Methyl orange indicator.*—Dissolve 0.2 g of methyl orange in 100 ml of  $\text{H}_2\text{O}$ .

### 14.23

#### DETERMINATION

Remove label and weigh sample bottle. Cool bottle and contents to 0° in refrigerator or in chopped ice and let stand overnight at this temp. Avoid shaking when removing bottle from refrigerator or chopped ice. Remove crown and immediately add 0.1–0.2 g of infusorial earth and 1–2 ml of hexyl alcohol. Quickly insert distilling head, outlet end of which is provided with rubber connection closed with screw clamp. Clamp bottle in position in empty liter beaker and connect with 250 ml tall Drechsel gas-washing bottle, which contains 25 ml of the KOH soln and 150 ml of  $\text{H}_2\text{O}$ . Open and adjust screw clamp to permit gas to pass into gas-washing bottle at rate of 3–4 bubbles/second. When screw clamp is completely open and gas evolution slows up, fill beaker with  $\text{H}_2\text{O}$  and apply full flame of burner. Keep  $\text{H}_2\text{O}$  in beaker boiling vigorously and add boiling  $\text{H}_2\text{O}$  to replace loss by evaporation. Continue until bubbles cease to come over and alkaline liquid in washing bottle rises in inner tube to level of outer liquid.

Disconnect gas-washing bottle, cool, and transfer contents with aid of  $\text{H}_2\text{O}$  to 500 ml Erlenmeyer flask. Titrate slowly, imparting rapid swirling motion to contents of flask, with the  $\text{H}_2\text{SO}_4$ , using 3 drops of the special phenolphthalein indicator, until lavender color of soln changes to phenolphthalein pink. Continue to faint pink and note buret reading (ml P. p.). Then add 2 drops of methyl orange indicator and con-

tinue titration to methyl orange end point (ml. M. O.). Apply blank correction for carbonates inherent in the KOH soln as determined by titrating, in above manner, 25 ml of the KOH soln in 150 ml of H<sub>2</sub>O to phenolphthalein end point and then to methyl orange end point. Difference (ml) between the two end points is the blank

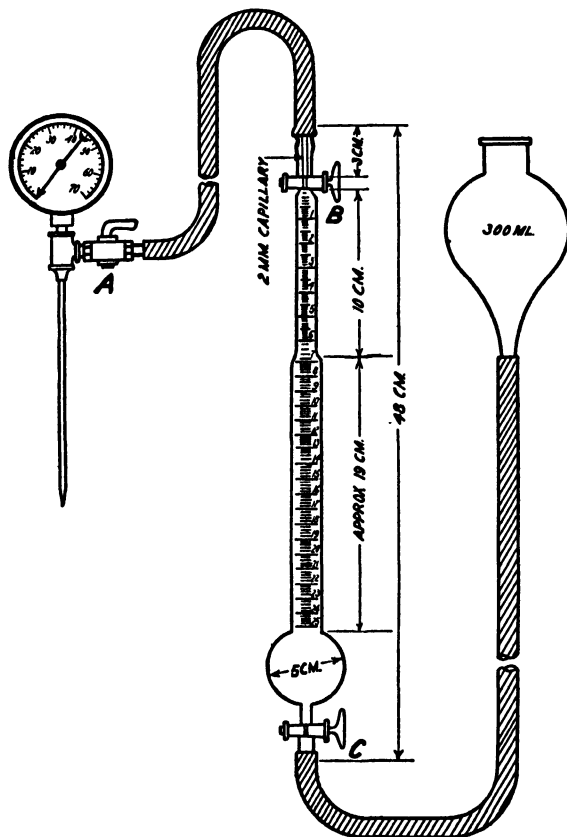


FIG. 19.—ABSORPTION BURET

correction (ml Blank). Disconnect beer bottle, wash, drain, and allow to dry. Weigh bottle and crown.

Calculate CO<sub>2</sub> as percentage by weight as follows:

Per cent CO<sub>2</sub> by weight

$$= \frac{(\text{ml M. O.} - \text{ml P. p.} - \text{ml Blank}) \times \text{titer of H}_2\text{SO}_4 \text{ (grams CO}_2\text{/ml)} \times 100}{\text{Weight of beer (grams)}}$$

Volumes CO<sub>2</sub> = % CO<sub>2</sub> by weight  $\times$  5.0607  $\times$  sp. gr. of beer.

Report CO<sub>2</sub> per cent by weight to second decimal and volumes CO<sub>2</sub> to first decimal.



*Manometric Method*

## 14.24

## APPARATUS

(a) *Piercing apparatus*.—(1) *For bottles*.—A gas-tight packing box and fastening for adjustment over crown of bottle, holding hollow-spike. (Suitable apparatus may be obtained from a number of manufacturers.) (2) *For cans*.—A metal frame, top of which is pressed or screwed down and locked over can top, holding hollow spike surrounded by compressible rubber sealing plug. The spike leads to accurate pressure gage and outlet valve. (One apparatus, adjustable for bottles and cans, may be used.)

(b) *Absorption buret*.—The buret (Fig. 19) consists of graduated tube (0–6 ml graduated in 0.1 ml divisions and 6–25 ml graduated in 0.2 ml divisions), having bulb, and closed at each end by stopcocks. Upper end is connected by rubber tubing to outlet valve of piercing apparatus and lower end is connected by length of rubber tubing to leveling bulb.

## 14.25

## DETERMINATION

If sample is in a bottle, make scratch mark at beer level; if sample is in a can, weigh can with contents. Submerge container in water bath at 25° long enough to bring temp. of beer to 25°. Connect piercing apparatus to the bottle or can. Fill absorption buret with 15% NaOH soln and allow soln to run up to stopcock B. Fill upper capillary of absorption bulb with hexyl alcohol and remainder of the system between B and the tip of the spike with H<sub>2</sub>O in order to displace any air. With outlet valve A closed, drive spike through crown or can top and thoroly shake and tap the bottle or can. Make pressure reading on gage. Again shake and take pressure reading. Use the pressure reading that shows no change in consecutive readings.

Open stopcocks B and C of absorption buret and then outlet valve A. Allow the gas, together with foam, to flow over into absorption buret. Swirl contents of buret to permit absorption of CO<sub>2</sub>. When  $\frac{1}{2}$ – $\frac{3}{4}$  of the alkali soln in absorption buret has been displaced, shut off all stopcocks and shake to permit complete absorption of CO<sub>2</sub>. Set buret in vertical position, open bottom stopcock, C, and allow alkali to flow back into bulb. Open stopcocks B and A and repeat above operation, tapping bottle or can to accelerate evolution of CO<sub>2</sub>. Close upper stopcocks A and B and shake thoroly to absorb last traces of CO<sub>2</sub>. Bring leveling bulb to such position that height of soln in leveling bulb and buret is same and read unabsorbed gas, which is reported as "air." Repeat operations until consecutive readings as "air" are same.

Disconnect bottle or can and determine head-space volume as follows:

If sample is a bottle, fill with H<sub>2</sub>O to top and pour off into graduated cylinder to scratch mark. Number of ml of H<sub>2</sub>O thus poured off represents head space in ml. If sample is a can, weigh empty can after pouring out all remaining beer. Difference represents weight of beer, which divided by sp. gr. of beer will give volume of beer in ml. Fill empty can with H<sub>2</sub>O and weigh. Weight of H<sub>2</sub>O in grams is also volume in ml, so that difference between volume of H<sub>2</sub>O and volume of beer represents head space in ml.

Calculate CO<sub>2</sub> by weight by following formula:

$$\% \text{CO}_2 = \left[ P - \left( \frac{\text{ml of "air"}}{\text{ml of head space}} \times 14.7 \right) \right] \times 0.00965, \text{ in which}$$

P = absolute pressure in lbs./sq. in. at 25° = (ordinary gage pressure + 14.7). (For routine work 15 may conveniently be substituted for 14.7. Pounds/sq. in.  $\times 0.070307$  = kg/sq. cm.)

Express CO<sub>2</sub> content in percent by volume, as:

$$\text{Volumes CO}_2 = \frac{\text{per cent CO}_2 \text{ by weight}}{0.1976} \times \text{sp. gr. of beer, or}$$

$$\text{Volumes CO}_2 = \text{per cent CO}_2 \text{ by weight} \times 5.0607 \times \text{sp. gr. of beer.}$$

Report percent by weight to second decimal place, and percent by volume to first decimal place.

## 14.26

## SULFUR DIOXIDE—OFFICIAL

Proceed as directed under 32.32, except to add thru dropping funnel 300 ml of beverage (not decarbonated), with no additional H<sub>2</sub>O, followed by 20 ml of HCl. Allow mixture to stand few minutes until fumes have settled. Adjust burner so that vapors rise no higher than one-tenth length of water jacket of condenser and boil sample 90 min. Adjust flow of CO<sub>2</sub> so that slow but steady stream passes thru receiver during distillation, and complete analysis as directed in 32.32. Report results as mg of SO<sub>2</sub>/liter.

## 14.27

## IODINE REACTION FOR UNCONVERTED STARCH

(a) *For light beer—Official, first action.*—(1) Place 10 ml of beer in one test tube, (2) in second test tube place 0.1 N I soln (dissolve 12.69 g of I and 25 g of KI in H<sub>2</sub>O and make to 1 liter) and dilute to same color as beer. Slowly pour the I soln into the beer and note color. A normal beer should not change in color. A blue color indicates starch; a purple color, amylopectine; and a reddish color, erythropectine. No change in color indicates complete conversion.

(b) *For dark beer, but applicable also to light beer—Tentative.*—To 5 ml of beer in test tube add 25 ml of alcohol. Shake thoroly. Let stand. Decant, pouring off last trace of the beer-alcohol mixture. Dissolve precipitate (pectine) in 5 ml of H<sub>2</sub>O and to this soln add dropwise a 0.1 N I soln diluted 5 times. See (a) for reaction.

## IRON—TENTATIVE

## 14.28

## REAGENTS

(a) *Nitric acid.*—Redistil thru Pyrex and store in Pyrex bottles.

(b) *Perchloric acid.*—Double-vacuum distilled 72% HClO<sub>4</sub>. Store in Pyrex bottles.

(c) *Soln of ammonium thiocyanate in methyl cellosolve.*—Dissolve 80 g of NH<sub>4</sub>SCN in methyl cellosolve and dilute to 2 liters with same solvent. (Faint pink color may be noticeable after reagent has been prepared, but this will fade upon standing.)

(d) *Standard iron soln.*—Contains equivalent of 0.01 mg of ferric Fe/ml. Add small quantity of Br water.

## 14.29

## PREPARATION OF STANDARDS

Make up standards by using H<sub>2</sub>O, HCl, and the NH<sub>4</sub>SCN soln in following proportions:

	ml
Standard Fe soln	X
H <sub>2</sub> O	5-X
HCl	1.25
NH <sub>4</sub> SCN soln	18.75

## 14.30

## DETERMINATION

Pipet 25 ml of thoroly degassed beer into 100 ml extraction flask (Pyrex No.

5160) and boil down to thick sirup, slightly charred. (It is necessary to use flask with rather small base so that the 2 ml of  $\text{HClO}_4$  that remains at end of digestion will cover entire bottom surface of flask.) Add 20–25 ml of the  $\text{HNO}_3$  and 2 ml of the  $\text{HClO}_4$ . Cover flask with watch-glass and heat gently until initial reaction begins, at which stage fairly vigorous boiling with the evolution of brown oxides of N takes place. After reaction has subsided, again heat contents of flask to slow boiling and continue boiling until all  $\text{HNO}_3$  is driven off. (With certain beers, or when too rapid boiling is used, this procedure may not complete the oxidation. In this case soln rapidly turns brown, or even black, depending on how much material remains unoxidized. A few additional ml of  $\text{HNO}_3$  at this stage will produce a clear but not colorless soln (brown).) Continue heating to expel all  $\text{HNO}_3$  as evidenced by evolution of copious fumes of  $\text{HClO}_4$ . (On cooling, residue should be colorless or at most pale yellow. If this is not the case, heat further with small additions of the  $\text{HNO}_3$  and  $\text{HClO}_4$ .)

After slight cooling, add 5 ml of  $\text{H}_2\text{O}$  and warm mixture until precipitated salts are in soln. Allow soln to cool to room temp. and add 18.75 ml of the  $\text{NH}_4\text{SCN}$ -methyl cellosolve reagent. Immediately after color is developed, place flasks and standards in pan of circulating cold  $\text{H}_2\text{O}$ , since heat causes slow fading of red color. Compare color produced in sample with that of standard of almost similar color intensity by means of colorimeter or photometer.

**14.31 OTHER METALS—TENTATIVE.—See Chap. 29**

**14.32 CHLORIDES—OFFICIAL**

Place 50 ml sample in Pt dish, add 20 ml of 5%  $\text{Na}_2\text{CO}_3$  soln, and proceed as directed under 12.42 or 12.44.

**14.33 END FERMENTATION AS INCREASE IN DEGREE OF FERMENTATION—TENTATIVE**

Ferment 250 ml of beer with active compressed brewers' yeast at 15–25° for 24–48 hours, or until fermentation is complete, providing fermentation flask with  $\text{H}_2\text{O}$  or Hg seal. Filter, determine real extract, 14.6, and calculate real degree of fermentation, 14.8. Increase in degree of fermentation is difference between real degree of fermentation and real degree of fermentation in the end fermented beer.

**14.34 COLORING MATTERS—TENTATIVE.—See Chap. 21**

**CARAMEL**

**14.35 Milos Test—Official.—See 15.38**

**14.36 Woodman-Newhall Test—Tentative.—See 21.16(f)**

**14.37 PRESERVATIVES AND SWEETENERS—OFFICIAL.—See Chap. 32**

**14.38 METHANOL (QUANTITATIVE)—TENTATIVE**

Transfer to distilling flask a quantity of sample that contains 20–25 ml of absolute alcohol and distil slowly, collecting distillate in 50 ml volumetric flask. When nearly to mark, disconnect receiver and adjust to mark at room temp. with  $\text{H}_2\text{O}$ . Determine methanol as directed under 16.29. See also 16.28 and 16.34.

**MALT**

**14.39 SAMPLING—TENTATIVE**

For complete descriptions of the trier, divider, sampler, and bushel weight tester, see "Handbook of Official Grain Standards of the United States Department of Agriculture" (1943).

(a) *Bulk malt in cars or bins.*—Using 60" trier, take at least 6 probes from different parts of car, preferably 2 from center and 2 from each end.

(b) *Bulk malt during discharge thru spouts or openings.*—At different times during filling or unloading of a car take, with the trier or sampler, at least 6 samples, each representing complete cross section from grain stream coming out of spout.

(c) *Bagged malt.*—Sample not less than 2% of bags, lengthwise thru center of the open bags, using short trier.

Indicate approximate proportion of inferior grain and take representative samples from each portion in manner outlined above. Immediately place each portion of sample in suitable large dry container and keep tightly closed.

## 14.40

## PREPARATION OF SAMPLE—TENTATIVE

Divide samples, either by quartering or by using sample divider, until ca 1 lb. remains. Place reduced sample in air-tight container (preferably tin with screw or friction type cover). Do not use cartons, bags, wooden boxes, glass Mason jars, or wrapping paper for this purpose. Remove foreign particles, such as stone, wood and twine. Do not remove foreign seeds or dust particles.

## 14.41

## BUSHEL WEIGHT—TENTATIVE

Place sample in filling hopper of Winchester tester, open slide underneath, and allow malt to fill measuring cylinder to overflowing. Without jarring, level off with straight-edge longer than diameter of measuring cylinder, making one forward stroke consisting of three distinct zig-zag motions. Weigh to nearest  $\frac{1}{2}$  pound.

## 14.42

*Alternative Method—Tentative*

(Not to be used to determine bushel weight for purpose of determining compliance with requirements of U. S. Grain Standards Act.)

Weigh 110 g of sample to nearest 0.1 g and pour evenly into metal funnel provided with plunger discharge and placed on top of 250 ml Normax cylinder graduated to meet N.B.S. specifications. (The funnel must fit snugly into the graduate and be sufficiently large to accommodate the grain without danger of spilling when the plunger is raised.) Then drop material into cylinder by pulling plunger up. Do not jar or tap the cylinder during this operation or before reading the volume, and do not read the very upper grain level, as compensation must be made for the ends of the few kernels that protrude. If the grain surface has a slant, repeat the test.

Make the calculation, which is based on the consideration that the Winchester bushel used in this country holds 2150.42 cu. in. or 35,239 ml, as follows:

$X = W/Y$ , where X represents bu. wt. in lbs., Y the volume in Winchester bushels, and W the weight in lbs. If weight is constant (K),  $X = K/Y$  or  $XY = K$ . To ascertain K, let bushel weight equal unity, then with the constant weight of sample in the test 110 g—

$$\frac{1 \text{ lb.}}{1 \text{ bu.}} = \frac{453.6 \text{ g}}{35,239 \text{ ml}} = \frac{110 \text{ g}}{8545 \text{ ml}} \cdot \text{Therefore } K = 8545, \text{ and bu. wt. in lbs.}$$

$$= \frac{8545}{\text{volume occupied by 110 g sample in ml}}$$

## 14.43

## LENGTH OF ACROSPIRE—TENTATIVE

For methods (a) and (b), quarter sample until ca 200 kernels remain in 2 opposite quarters, and count out 100 kernels, rejecting those that are broken or those in which growth is not ascertainable.

(a) *Cutting.*—Hold each kernel furrow downward upon flat surface with pair of

tweezers, cut thru kernel longitudinally with razor blade or other sharp instrument, and examine cut acrospire in both halves, comparing its length with that of the kernel. Tally according to classifications below.

(b) *Peeling*.—Remove husk covering acrospire by means of sharp instrument and examine acrospire length in comparison with length of kernel. Tally according to classifications below.

(c) *Boiling*.—Boil 10–15 g of average sample with 75–100 ml of H<sub>2</sub>O. After boiling, add cold H<sub>2</sub>O to cool contents of beaker. Decant, and pour grain on glass plate. Count 100 kernels at random, inspect acrospire, and tally according to classifications below.

Classify kernels as follows and report percentage in each group:

0– $\frac{1}{4}$ —includes those kernels without apparent growth, or having an acrospire development up to, but not including  $\frac{1}{4}$  length of grain.

$\frac{1}{4}$ – $\frac{1}{2}$ —includes those kernels having acrospire development from  $\frac{1}{4}$  up to, but not including  $\frac{1}{2}$  length of grain.

$\frac{1}{2}$ – $\frac{3}{4}$ —includes those kernels having acrospire development from  $\frac{1}{2}$  up to, but not including  $\frac{3}{4}$  length of grain.

$\frac{3}{4}$ –1—includes those kernels having an acrospire development of  $\frac{3}{4}$  but not greater than entire length of grain.

Overgrown—includes those kernels having acrospire development in excess of length of grain.

Where it is apparent that an overgrown acrospire has been broken off during processing, the kernel shall be included in the overgrown classification regardless of length of remaining stub.

#### 14.44

#### MEALINESS—TENTATIVE

Count out 100 kernels remaining from preceding test if method (a) or (b) was used. Otherwise select 100 kernels as described in 14.43. Determine percentage of mealy, half glassy, and glassy kernels. In case of uncertainty, pierce starch body with sharp point; if mealy, it will break away and crumble from the point.

#### 14.45

#### 1,000 KERNEL WEIGHT—TENTATIVE

Quarter sample until ca 500 kernels remain in two opposite quarters. Count out 500 kernels and weigh to nearest 0.1 g. Calculate results to 1,000 kernels on “as is” and dry basis.

#### 14.46

#### ASSORTMENT\*—TENTATIVE

Weigh 100 g from a quartered sample to nearest 0.1 g. Place in top compartment of grader and shake 3 min. ( $\pm 30$  sec.). Weigh quantities remaining on various screens and in catch pan to nearest 0.1 g, and report percentage on each of following screens: 7/64", 6/64", 5/64", and thru 5/64", in percentages totaling 100%. (When testing large berried malts (2 row, California, etc.) addition of 8/64" screen is optional.)

#### 14.47

#### MOLD—TENTATIVE

Determine presence or absence of mold by visual inspection and report as “none,” “trace,” etc.

#### 14.48

#### FOREIGN SEEDS AND BROKEN KERNELS—TENTATIVE

Weigh 50 g of sample. Pick out foreign seeds and broken kernels, classify, and report separately in percentage.

\* Grader frame without screens is manufactured by the Richmond Mfg. Co., Lockport, N. Y. Standard screens can be obtained from the American Society of Brewing Chemists.

## MOISTURE—OFFICIAL, FIRST ACTION

## 14.49

## APPARATUS

(a) *Weighing dish*.—Use glass bottle, or an Al dish, provided with tight fitting cover and having diameter of ca 40 mm for a 5 g sample, or 55 mm for 10 g sample.

(b) *Oven*.—Should have automatic temp. control capable of holding temp. within  $\pm 0.5^\circ$ , and be large enough to accommodate all samples on one shelf in such manner that no sample is outside area indicated by test to give comparable results in duplicate samples. Standardize oven as follows: Place weighed duplicate samples in oven at  $103\text{--}104^\circ$  and dry 3 hours. Weigh, and redry 1 hour longer. If loss of moisture is more than 0.1%, raise temp.  $1^\circ$  and again test with new duplicate samples. Take as standard lowest temp. below  $106^\circ$  giving moisture content which, after 3 hours of drying, is within 0.1% of value attainable at same temp. within 4 hours. Keep ventilators of oven open during entire drying period, and do not open door during the 3 hours of drying.

## 14.50

## PREPARATION OF SAMPLE

(a) *If extract determination is to be made*.—Grind as instructed under 14.56, and transfer in one continuous operation. When many samples are to be analyzed, grind first sample, remove beaker, and grind second sample while adjusting weight of first sample. Remove second sample, insert third sample, and repeat operation.

(b) *If extract determination is not to be made*.—Have sample of same fineness as the finely ground malt used for determination of extract. Weigh ca 5 g of whole malt and grind thru clean dry mill directly into weighing bottle (10 g may be taken provided the 55 mm diameter weighing bottle is used). Brush all malt from mill into weighing bottle and cover immediately.

## 14.51

## DETERMINATION

Weigh sample to 0.1 mg and place in oven previously heated to standard temp. Remove cover of weighing bottle and heat exactly 3 hours at the standard temp. Transfer weighing bottle, with cover replaced, to desiccator and when cooled to room temp. weigh to 0.1 mg. Report moisture to nearest 0.1%

## 14.52

## MOISTURE IN CARAMEL MALT AND BLACK MALT—TENTATIVE

Proceed as directed in 14.50 (a) or (b) and 14.51.

## EXTRACT—OFFICIAL

## 14.53

## REAGENT

*Iodine solns.*—(a) 0.01 N. Dissolve 0.63 g of I and 1.25 g of KI in  $\text{H}_2\text{O}$  and make to 500 ml. (b) 0.02 N. Dissolve 1.27 g of I and 2.50 g of KI in  $\text{H}_2\text{O}$  and make to 500 ml. Prepare fresh solns every month.

## 14.54

## APPARATUS

(a) *Mill*.—Miag-Seck. For fine grinding use the cone-type, 300 r.p.m., and for coarse grinding the roller-type, 150 r.p.m.

(b) *Sieves*.—Half-height, 8" U. S. standard sieve No. 30 (with pan and cover). For classification of laboratory and brewery grindings use additional U. S. standard sieves Nos. 10, 14, 18, 60, and 100.

(c) *Mash beakers and counter weights*.—Made of either pure Ni or brass, not Cu, and of such dimensions as to assure tight connection between beakers and the Miag-Seck mill during grinding period.

If counter weights are used for the mash beakers, check their tare weight frequently.

(d) *Mashing apparatus*.—Beakers, stirrers, and solder shall be made of the same metal. Each stirrer shall be provided with a blade, which in operation has clearance of ca 2 mm from bottom and 5 mm from wall of mash beaker. The blade shall be ca 8 mm wide, and each side shall have a pitch of 45°, arranged as in a propeller, to cause an upward motion of mash. The speed of mash stirrer shall be 80–100 r.p.m., each stirrer of each beaker having the same speed. Stir the H<sub>2</sub>O in bath thoroly by mechanical means to assure uniformity of temp, and have level of H<sub>2</sub>O above maximum mash level.

(e) *Gypsum plate*.—Prepare by thoroly mixing 100 ml of H<sub>2</sub>O with 135 g of plaster of Paris. Pour this mixture, while still free-flowing, into suitable flat molds (cigar boxes, etc.). Porcelain plate for color reactions, having dimensions 118×91×7 mm, may be used.

(f) *Filter paper*.—Use Schleicher and Schüll 32 cm fluted filter paper No. 560 (or No. 597, 32 cm, to be fluted by analyst) or Delta Düren, 32 cm fluted filter paper No. 314½ (or No. 314, 32 cm, to be fluted by analyst), or Eaton-Dikeman, 32 cm, fluted filter paper No. 509 (or No. 609, 32 cm, to be fluted by analyst).

(g) *Funnels*.—Use short-stemmed glass funnels of ca 20 cm diameter and do not allow filter paper to project above rim of funnel. Stem shall extend 3–5 cm into receiving flask.

(h) *Flasks*.—Use dry 500 ml Erlenmeyer flasks. Mark at 100 ml level.

(i) *Pycnometers*.—Use any suitable pycnometer, but preferably Reischauer or Boot (vacuum) type. The Reischauer type is ca 15 cm high and has neck ca 9 cm long and internal neck diameter between 2.5 and 3.5 mm. Place thin, well defined mark 55–70 mm below upper rim of neck. When filled with H<sub>2</sub>O at 20° it shall have capacity of 48–50 g. Use glass funnels having capacity of ca 15 ml to fill pycnometers.

The Boot type is of cylindrical shape and holds ca 50 g of H<sub>2</sub>O at 20°. The vacuum seal is well rounded off and not pointed. The pycnometer opening is sufficiently wide to permit easy filling and emptying and the stopper has fine capillary opening. The walls of bottle meet stopper in rising acute angle of ca 45° so that no depression or groove retaining moisture is formed at this point.

(j) *Emptying device for Reischauer pycnometer*.—Bend piece of metal capillary tubing (brass, stainless steel) of less than 2 mm outside diameter to angle of ca 45°. End to be inserted into pycnometer shall reach within few mm of bottom. Connect other end either to rubber aspirator bulb or to compressed air supply not exceeding 5 lbs./sq. in.

(k) *Water bath*.—Automatically controlled water bath. If automatic control is not available, use following set-up: Have water level of bath (5–15 liters capacity) reach above neck marks of pycnometer, keep water bath temp. at 20° (±0.05°), and read on accurate thermometer, calibrated to 1/10°. Maintain temp. of water bath by very slow but continuous flow of ice water from container (2–4 liters capacity, containing ice and H<sub>2</sub>O). Regulate flow of ice water by hand. Stir H<sub>2</sub>O in bath mechanically and continuously without splashing.

## 14.55

## STANDARDIZATION

(a) *Setting of mill*.—Use malt of following characteristics:

Moisture.....	4.0–5.0%
Extract in finely ground malt (Plato) as is.....	68–71%
Color of laboratory wort, Lovibond series 52-½" cell ...	1.5–2.2°
1000 kernel weight, as is.....	22–26 g

Acrospire development: 80% of kernels to have an acrospire longer than  $\frac{1}{2}$  of kernel length; not more than 5% of kernels to have acrospires longer than kernel length.

*Fine grinding.*—Weigh ca 51 g of specified malt into mash beaker, grind, and collect in same beaker. Sieve 50 g of well-mixed ground malt thru standard sieves. Shake set of sieves with malt by hand in horizontal plane 5 min., tapping upon table top every 15 seconds. Remove pan and cover and shake for short time over sheet of paper until no particles are emitted. The mill shall be considered as having standardized setting when portion of ground malt remaining on sieve No. 30 is between 4.5 and 5.5 g (9–11%). Standardize mill at least twice yearly. A suitable mechanical shaking device, giving equivalent results, may be used for standardization of mill.

*Coarse grinding.*—Proceed as directed for *fine grinding*. The mill shall be considered as having standardized setting when portion of ground malt remaining on sieve 30 is between 36.5 and 38.5 g (73–78%).

(b) *Reischauer type pycnometer.*—Clean interior and exterior of pycnometer with  $\text{Na}_2\text{Cr}_2\text{O}_7\text{--H}_2\text{SO}_4$  soln, discharge carefully with air, and wash several times with  $\text{H}_2\text{O}$ , then with alcohol and finally with ether. To remove last traces of ether vapor and replace it with laboratory air, connect metal capillary tubing (dry) to vacuum and insert into pycnometer 1–2 min. Carefully wipe pycnometer, allow to stand a few minutes, and determine tare by weighing to 0.2 mg.

Fill with freshly distilled  $\text{H}_2\text{O}$  and place in water bath held at  $20^\circ (\pm 0.05^\circ)$ . Force out air bubbles by gentle tapping. After 25 min., remove liquid above mark by means of capillary pipet provided with small rubber bulb. Make final adjustment of meniscus by absorbing last quantity of liquid by means of thin strips of blotting paper; also remove any liquid adhering to inner surface of neck. Adjust water level so that lower part of meniscus rests on mark. Make all adjustments of liquid level within pycnometer neck while holding by neck, without touching body of pycnometer with hands. Keep body of pycnometer submerged during entire period of meniscus adjustment.

Raise pycnometer to room temp. by insertion into water bath kept at exactly that temp., and hold 10 min. Remove pycnometer, carefully dry exterior, and weigh to 0.2 mg. The difference between the two weighings represents  $\text{H}_2\text{O}$  capacity of the pycnometer at  $20^\circ$ . Redetermine tare weight and  $\text{H}_2\text{O}$  capacity at least twice a year.

(c) *Boot type pycnometer.*—Clean pycnometer and determine its tare in same manner as for Reischauer type. Cool  $\text{H}_2\text{O}$  in ice bath to a temp. somewhat below  $20^\circ$ . Rinse pycnometer once with the cool  $\text{H}_2\text{O}$ , fill, stopper, and dry exterior. Remove stopper, insert thermometer, adjusted to  $20^\circ$  in  $\text{H}_2\text{O}$ , and ascertain temp., which should be  $20^\circ (\pm 0.1^\circ)$ . If it is not, choose different “filling” temp., which may vary with analyst and season from  $19.4^\circ$  to  $19.7^\circ$  or more. Make subsequent determinations, using the pre-determined filling temp. Place cap over stopper and weigh.

#### 14.56

#### DETERMINATION

*Fine grinding.*—Weigh ca 55 g of sample (at room temp.) into tared mash beaker and grind thru mill set for standardized fineness of grind. Collect finely ground malt in same mash beaker, carefully brushing malt particles remaining in mill into mash beaker. Without delay, place mash beaker with contents on balance (accurate to within  $\pm 0.05$  g under 750 g load) and adjust weight of malt to 50 g ( $\pm 0.05$  g) by removing excess into tared dish for determination of moisture.

*Coarse grinding.*—Weigh 50.5 g or less of sample (at room temp.) into tared mash



beaker and grind thru mill, set for standardized coarseness of grind. Collect coarsely ground malt in same mash beaker, carefully brushing particles remaining in mill into mash beaker. Without delay, place mash beaker with contents on balance (accurate to within  $\pm 0.05$  g under 750 g load) and adjust weight of malt to 50 g ( $\pm 0.05$  g) by removing excess.

(a) *Mashing procedure*.—"Mash in" ground malt with 200 ml of  $H_2O$  at  $46^\circ$  and mix well with glass rod to prevent formation of lumps. Carefully rinse glass rod and wall of beaker with small quantity of  $H_2O$ . Note odor of mash and report as aromatic, slightly aromatic, musty, green, stale, etc. Promptly place mash beakers in mashing apparatus containing  $H_2O$  previously heated to  $46^\circ$ , and set stirrers in motion. Place thermometer in each mash beaker. Maintain temp. of  $45^\circ$  exactly 30 min. from time beakers were placed in mashing apparatus. Raise mash temp.  $1^\circ/\text{min.}$  until  $70^\circ$  is reached. Add 100 ml of  $H_2O$ , previously heated to  $70\text{--}71^\circ$ , and hold mash at  $70^\circ$  60 min. (Temp. deviations during mashing procedure should not exceed  $0.5^\circ$ .)

(b) *Conversion*.—Transfer drop of mash by means of thin glass rod (ca 3 mm diameter) onto absorbent gypsum plate, or into one of the cavities of the porcelain plate, and test with drop of 0.01 *N* I soln on the gypsum plate, or with a drop of 0.02 *N* I soln, 14.53(b), on the porcelain plate. Make tests 5, 7 and 10 min. after  $70^\circ$  has been reached, and thereafter, if necessary, at 5 min. intervals. Conversion is complete when test drop and I produce only yellow stain on gypsum or porcelain plate. Report time of conversion in periods: less than 5 min., 5–7 min., etc. Time of conversion is not determined on coarsely ground malt.

(c) *Cooling and filtration*.—Cool mash promptly (within 10–15 min.) to prevailing room temp. Stop stirrers. Remove thermometers after adhering mash particles have been rinsed into beaker with  $H_2O$ . Remove each beaker with its stirrer from mashing apparatus. Rinse mash particles adhering to stirrer into beaker with  $H_2O$ . Dry outside of each beaker, taking care to remove moisture adhering to rim. Without delay, adjust weight of contents of mash beaker to 450.0 g ( $\pm 0.05$  g) by addition of  $H_2O$ .

Stir mash thoroly with glass rod, once when removing beakers from balance pan, and again immediately before pouring mash onto filter. (The two stirrings must be not less than 5 min. nor more than 15 min. apart.) During stirring of cooled mash, take care to prevent splashing or spilling. Mix drops adhering to beaker wall into mash by rotary stirring with glass rod.

Pour entire contents of beaker into funnel provided with specified filter paper. Cover funnel with large watch-glass (ca 20 cm diameter) during entire filtration. Return first 100 ml of filtrate to filter. When no more liquid is present above filter cake, discontinue filtration and remove receiving flask containing wort for subsequent observations and tests. In case of slow running worts, stop filtration after 2 hours. When filtration is complete, mix wort in receiving flask thoroly by rotary motion. Speed of filtration is normal if filtration is complete (as defined above) within 1 hour after returning the 100 ml filtrate to filter bed; slow, if filtration requires longer time. Observe degree of clarity and report as clear, slightly hazy, or hazy.

Remove ca 100 ml of wort for determination of color. Color is not determined on wort from coarsely ground malt.

(d) *Specific gravity*.—Rinse empty pycnometer twice with ca 10 ml of wort, and if Reischauer pycnometer is used remove rinsings each time by means of emptying device. Fill with wort, place in water bath, and follow procedure under 14.55(b). Weigh filled pycnometer within 3 hours of completed filtration. Difference between this weight and that of empty pycnometer represents wort capacity of pycnometer

at 20°. Calculate sp. gr. of wort to fifth decimal place, rounding off to 0.00005 or 0.00010, by dividing weight of wort by weight of H<sub>2</sub>O.

No calculation is made of sp. gr. in vacuo. If duplicate determinations made by same analyst in different beakers differ by more than two units in fourth decimal place, repeat entire determination.

(e) *Extract*.—Ascertain extract yield of wort by reference to sp. gr. values given in 44.3, and calculate extract yield of malt by following formulas:

Extract "as is" =  $\frac{P(800+M)}{100-P}$ , in which  $P$  = extract in 100 g of wort (Plato, 44.3);

and  $M$  = % H<sub>2</sub>O in the malt.

Extract "dry basis" =  $\frac{E \times 100}{100-M}$ , where  $E$  = extract "as is"; and  $M$  = % H<sub>2</sub>O in the malt.

Report extract "as is" and on "dry basis" only to first decimal place.

#### 14.57

#### EXTRACT IN CARAMEL MALT—TENTATIVE

For grinding of sample use a mill for fine grinding as directed under 14.54(a).

Weigh ca 25.5 g of caramel malt, grind, and adjust to 25 g ( $\pm 0.05$  g) by removing excess.

Weigh ca 25.5 g of malt of known moisture, extract, and color and having diastatic power of 100° L. or over; grind, and adjust to 25 g ( $\pm 0.05$  g).

Transfer quantitatively the two portions to mash beaker, mash, and determine sp. gr. as directed under 14.56.

Determine moisture as directed under 14.50(b) and 14.51.

Calculate yield by following formulas:

$$\text{Total extract} = \frac{P \times (800 + M \text{ in 50 g of malt} + M \text{ in 50 g of caramel malt})}{100 - P},$$

where  $P$  = g of extract in 100 g of wort (Plato), and

$M$  = moisture in g.

$$\text{Extract in caramel malt} = \frac{(\text{Total Extract} - \text{Extract in 5 g of malt}) \times 100}{50}.$$

#### 14.58

#### COLOR IN CARAMEL MALT—TENTATIVE

Use mixed wort obtained for extract determination, diluting wort sufficiently to make color reading ca 4.0° L. Determine color on diluted wort in a  $\frac{1}{2}$ " cell with Lovibond tintometer as directed under 14.55.

Calculate color by the following formula:

Color of caramel malt =  $2[L \times (D + 1)]$  - color of malt used for conversion,

where  $L$  = color reading on diluted wort, and

$D$  = parts of H<sub>2</sub>O used to dilute one part of wort.

Report dilution used for making color reading.

#### 14.59

#### COLOR IN BLACK MALT—TENTATIVE

For grinding of sample use mill for fine grinding as directed under 14.54(a). As precautionary measure grind small quantity of sample to be analyzed and clean out mill. For the determination weigh 5.5 g, grind, and collect all particles by careful brushing of mill.

Weigh 5.00 g on analytical balance, transfer to glass beaker, add 400 ml of  $H_2O$  at room temp., and heat to boiling. Boil gently for exactly 5 min., cool to room temp., and without delay transfer to 500 ml volumetric flask; make to volume with  $H_2O$ , mix, and filter. Pipet 10 ml of filtrate into 100 ml volumetric flask, make to volume with  $H_2O$ , and mix.

Determine color of diluted filtrate in a  $\frac{1}{2}$ " cell, using Lovibond tintometer as specified under 14.60. Calculate color found for this filtrate to same concentration of materials as is used for regular malt mash (12.5 g of malt to 100 ml of  $H_2O$ ) by formula:

Color of black malt =  $L \times 10 \times 12.5$ , where  $L$  = color reading on diluted filtrate. Report color to nearest whole number.

## 14.60

## COLOR OF WORT—OFFICIAL, FIRST ACTION

Use Lovibond tintometer,  $\frac{1}{2}$ " cell, Series 52, brewers' type, and standard daylight lamp (A.S.T.M., D 218-34T, 1933, or its spectrophotometric equivalent). Place tintometer in box shield of metal or wood, finished in dull black so as to prevent interference from reflected light. Mount in horizontal position directly in front of artificial daylight lamp. Substitute flashed opal glass for milk glass usually provided with instrument. Have distance between opal glass and daylight lamp such as to project diffused light with absence of glare or shadow upon opal glass and have near surface of daylight filter 6" from opal glass.

Pour wort into cell as quickly as possible after filtration and match against the standard glasses. Subdivide down to  $\frac{1}{4}^\circ$  color glasses and report results to nearest tenth. If difficulty is experienced in reading color, filter that portion of wort to be used for color determination separately thru dry filter paper without filter-aid.

## DIASTATIC POWER—TENTATIVE

## 14.61

## PREPARATION OF GLASSWARE

Wash all glassware with acid cleaning soln, 14.55(b), then rinse with ordinary tap  $H_2O$  not less than 4 times, and finally rinse with distilled  $H_2O$  at least twice. Thoroughly dry digestion flasks.

## 14.62

## REAGENTS

(a) *Acetate buffer soln.*—Dissolve 68 g of Na acetate ( $CH_3COONa \cdot 3H_2O$ ) in 500 ml of normal acetic acid and make to 1 liter with  $H_2O$ .

(b) *Fehling soln.*—Standardize as directed in 34.34, 34.35. Check soln from time to time by estimating its oxidizing value against a standard soln of invert sugar according to customary analytical procedure.

(c) *Alkaline ferricyanide soln.*—See 20.61(b).

(d) *Sodium thiosulfate soln.*—See 20.61(c).

(e) *Acetic acid soln.*—See 20.61(d).

(f) *Potassium iodide soln.*—See 20.61(e).

(g) *Starch soln.*—Have final concentration represent 2 g of soluble starch (weighed on dry basis) in 100 ml of soln. Use starch of such quality and grade that its solubility shall be at least 1:50 in hot  $H_2O$ , that it shall contain no dextrines, contain less than 0.75% reducing substances calculated as maltose, and have moisture content of 10–12%. A freshly made 2% soln shall have a pH of 4.5–5.5 without adjustment by use of a buffer. Subsequent batches of starch shall, when tested on a malt of ca 100° Lintner (dry basis) having other characteristics as specified under determination of extract in malt, show a variation no greater than  $\pm 3^\circ$  Lintner from value obtained by using original starch in parallel determination. Further additional batches of starch when purchased shall be tested in parallel with starch in use. No variation greater than  $\pm 3^\circ$  Lintner shall be permitted. In no case shall a cumulative

correction as referred to original starch, approved above, amount to more than 5° Lintner.\* Macerate starch with only sufficient cold freshly distilled H<sub>2</sub>O to form smooth thin paste (not over 5% of final volume). Pour this, with constant stirring, into boiling freshly distilled H<sub>2</sub>O representing not less than ca 75% of final volume of starch soln, at such rate that boiling does not cease. Continue boiling 2 min. after thin paste is completely introduced. Quickly add to beaker an additional 10% of final volume of cold freshly distilled H<sub>2</sub>O and transfer mixture quantitatively to glass-stoppered volumetric flask; mix by inverting flask, wash down neck of flask, and cool to 20° before adding buffer soln. Add 2 ml of the buffer soln for each 100 ml of final volume of starch soln and make to mark. Mix again by inverting flask and keep tightly stoppered at 20° until used.

## 14.63

## DETERMINATION

Grind separately not over 25.5 g of malt as directed under 14.56. Collect finely ground malt in mash beaker, carefully brushing in malt particles remaining in mill. Without delay, adjust weight of contents to 25 g ( $\pm 0.05$  g). Transfer quantitatively to container (capacity ca 1 liter) in which infusion is to be made. Add 500 ml of freshly distilled H<sub>2</sub>O and close container. Let infusion stand 2.5 hours at 20° ( $\pm 0.2^\circ$ ) and agitate by rotation at 20 min. intervals. Take care that in agitation of malt suspension as small a quantity as possible of grist is left adhering to inner surface of flask above level of the H<sub>2</sub>O. (Mixing by inverting the flask is specifically cautioned against; gentle whirling of contents without splashing on sides of container has been found to be sufficient.) Filter infusion by transferring entire charge to 30–32 cm fluted filter (CS and S No. 588 or its equal in quality) contained in 175 mm funnel. Return first 50 ml of filtrate to filter. Collect filtrate until 3 hours shall have elapsed from time the H<sub>2</sub>O and ground malt were first mixed. Prevent evaporation during filtration period as far as possible by placing watch-glass over funnel and some suitable cover around stem of funnel, resting on neck of receiver.

Immediately dilute 20 ml of above infusion to 100 ml at 20°, transfer 10 ml of this infusion to 250 ml volumetric flask, and bring to 20°. Add 200 ml of buffered starch soln from fast-flowing pipet, all at 20°. Mix soln by rotating flask during addition. Maintain the "starch infusion" mixture at 20° ( $\pm 0.2^\circ$ ) for exactly 30 min., timed on stop-watch from time addition of starch was begun. Add 20 ml of 0.5 N NaOH rapidly and mix well by whirling the flask. Make to mark at 20° and mix thoroly.

Determine reducing power of (a) Fehling soln modification, or (b) the ferricyanide modification.

(a) *Fehling soln modification*.—Boil 10 ml of the Fehling soln and 10 ml of H<sub>2</sub>O in small flask with narrow neck (200 ml Erlenmeyer). (For heating soln, electric plate is preferable to gas flame.) Add from buret ca  $\frac{1}{3}$  of amount of above digested starch soln probably required and boil 15–20 seconds, rotating constantly. Remove from heat source. If still decidedly blue, add more soln, boil ca 10 seconds, and again observe color. When blue color has been almost discharged, and after boiling gently ca 2 min., add 3 drops of 1% aqueous methylene blue soln. Continue boiling and add more soln until 0.1 ml, or even 1 drop, upon boiling, discharges the blue color. (Color becomes violet-lavender as end point nears.)

Repeat titration, adding at once almost whole amount of digested starch required, and proceed to end point as directed. Let amount of digested starch soln required to reach end point in this second titration be called *A*. Interrupt boiling as little as possible after indicator has been added, so that flask remains filled with steam, preventing much access of air. (Upon cooling, blue color usually returns.)

Prepare blank by processing exactly as directed above, except to add the 0.5 N

\* Starch meeting these specifications is available from Merck & Co., Rahway, N. J., and from J. T. Baker Chemical Co., Phillipsburg, N. J.

NaOH to malt infusion before adding the starch soln. Add to 10 ml of the Fehling soln and 10 ml of  $H_2O$  a volume of this blank soln equal to final volume of digested starch soln required in above determination. Boil and again determine end point as in the determination. Let amount of digested starch soln used be called *B*.

(b) *Ferricyanide modification*.—Proceed as directed in 20.62, beginning "pipet 5 ml of filtered extract" thru "disappearance of blue color (10 ml buret is recommended)."

Subtract number of ml of 0.05 *N*  $Na_2S_2O_3$  used in titration (*A*) from number of ml obtained in titrating the blank (*B*).

## 14.64

## CALCULATION OF DIASTATIC POWER

(a) *Fehling soln modification*.—Degrees Lintner ( $^{\circ}L$ ) (as is) =  $\frac{5000}{A} \times \frac{B}{A}$ ;

$^{\circ}L$  (dry basis) =  $\frac{^{\circ}L \text{ (as is)} \times 100}{100 - M}$ , where *A* and *B* have same meaning as in 14.63(a),

and *M* = per cent moisture in sample.

In above formula, 5000/*A* is apparent diastatic power which must be modified by fraction representing ratio of blank titration to original titration, which measures influence of the starch in the determination.

Report  $^{\circ}L$  "as is" and "dry basis" to nearest whole number.  $^{\circ}L \times 4$  = Maltose Equivalent (M.E.) (g of reducing substances, calculated as maltose, produced by 100 g of malt in half-hour digestion of soluble starch at 20° under conditions specified in method).

M.E. (as is) =  $\frac{20,000}{A} \times \frac{B}{A}$ ; M.E. (dry basis) =  $\frac{M.E. \text{ (as is)} \times 100}{100 - M}$ ; where *A*, *B*,

and *M* are as defined above.

(b) *Ferricyanide modification*.—Degrees Lintner ( $^{\circ}L$ ) (as is) =  $(B - A) \times 23$ , where *A* and *B* have same meaning as in 14.63(b). Calculate  $^{\circ}L$  (dry basis) from this as directed under (a) and report to nearest whole number.

Maltose Equivalent (M.E.) (as is) =  $(B - A) \times 92$ . Calculate M.E. (dry basis) from this as directed under (a).  $\frac{M.E.}{4} = ^{\circ}L$ .

(c) *Ferricyanide equivalent*.—When conditions are maintained as set forth in method, net amount of ferricyanide, after correcting for blank, multiplied by 23, gives diastatic power in Degrees Lintner (as is). Degrees Lintner  $\times 4$  = Maltose Equivalent. Therefore Ferricyanide Equivalent  $\times 92$  = Maltose Equivalent.

## CEREAL ADJUNCTS—OFFICIAL, FIRST ACTION

## 14.65

SAMPLING.—See 14.39(a), (b) and (c)

## 14.66

PREPARATION OF SAMPLE.—See 14.40

## 14.67

## PHYSICAL CHARACTERISTICS

(a) *Removal of accidental foreign particles*.—Before proceeding with laboratory determinations remove any accidental particles from sample.

(b) *Color*.—Spread evenly suitable portion of sample and observe against white background. Report as white, cream, buff, gray, brown.

(c) *Odor*.—Determine after shaking sample in closed container. Report as clean and normal, musty, rancid.

(d) *Husks, germs, yellow corn, and foreign seeds*.—Classify and report in percentage.

(e) *Mold*.—Determine by visual inspection and report as none, trace, considerable.

(f) *Weevils, larvae, etc.*—Determine their presence or absence by visual inspection. Report as none, very few, few, considerable.

#### MOISTURE

*Air oven method (103–104°)*

14.68

APPARATUS—See 14.49

14.69

DETERMINATION

Grind as directed under 14.56 and proceed as directed under 14.51.

#### OIL OR PETROLEUM BENZINE EXTRACT

14.70

REAGENT

*Petroleum benzine*.—See 31.66.

14.71

DETERMINATION

Use accurately weighed sample (5–10 g) ground as described under 14.56. Without previous drying, extract in Soxhlet or other suitable extractor with the petroleum benzine for not less than 6 hours. Filter extract thru small, hardened filter paper into weighed vessel, washing filter paper finally with small portion of hot fresh solvent. Distil or evaporate solvent at temp. not exceeding 100° and dry vessel containing residual extractive matter in hot air oven at temp. of 100–105° for 1 hour. Report as % oil to second decimal.

#### EXTRACT

14.72

APPARATUS

Same as under 14.54 except that mill may be of any suitable type.

14.73

STANDARDIZATION

*Setting of mill*.—Use sample of rice or grits the moisture content of which is not over 12%. Grind sufficient quantity of sample so that at least 51 g of ground portion is obtained. Determine fineness of grinding as directed under 14.55(a). Fine grinding of rice should show  $40 \pm 2.5$  g ( $= 80 \pm 5\%$ ) and grits  $35 \pm 2.5$  g ( $= 70 \pm 5\%$ ) of ground portion passing thru standard sieve.

14.74

DETERMINATION

Grind sufficient amount of sample so that at least 21 g is obtained. Grind ca 31 g of a malt made mainly from six-rowed barley of Manchurian type, conversion time not longer than 7 min., diastatic power 100–120° Lintner. Determine extract of the malt simultaneously with that of the cereal. Mash in 20 g ( $\pm 0.05$  g) of sample (with exception of flaked corn and flaked rice) and 5 g ( $\pm 0.05$  g) of the ground malt with 200 ml of H<sub>2</sub>O at 46°. Mix well with glass rod, place on wire gauze over flame,

and bring to boiling within 15 min. with constant stirring. Boil grits and rice gently for half an hour, and refined grits for 10 min., avoiding burning, spattering, and excessive frothing. During boiling stir mash and keep volume constant by adding boiling  $H_2O$  every 15 min. Upon completion of boiling, cool to  $46^\circ$  and add 25 g ( $\pm 0.05$  g) of the remaining ground malt. When extract is determined on flaked corn or flaked rice, do not boil, but mash in 20 g of the unground sample and 30 g of the ground malt with 200 ml of  $H_2O$  at  $46^\circ$ . Mix well to prevent formation of lumps, rinse inner walls of beaker, promptly place mash beakers in mashing apparatus containing  $H_2O$  previously heated to  $46^\circ$ , and set stirrers in motion. Hold at  $45^\circ$  for 30 min. from time mash beakers were placed in apparatus. Raise mash temp.  $1^\circ/\text{min.}$  until  $70^\circ$  is reached. Add 100 ml of  $H_2O$ , previously heated to  $70-71^\circ$ , and hold mash at  $70^\circ$  for 30 min. (All temps. refer to mash, not water bath temp. Temp. deviation during mashing should not exceed  $0.5^\circ$ .) To test conversion, transfer a drop of mash to one of cavities of porcelain plate and add drop of 0.02  $N$  I soln, 14.53(b); conversion is completed when test drop and I give yellow color. Report time of conversion in periods: less than 15 min., 15-30, 30-45, 45-60, incomplete at 60 min. Cool, filter as directed under 14.56(c), and determine sp. gr. and corresponding extract as directed under 14.56(d) and (e).

## 14.75

## CALCULATION

$$\text{Total extract} = \frac{P \times (800 + M \text{ in } 60 \text{ g malt} + M \text{ in } 40 \text{ g sample})}{100 - P}, \text{ where}$$

$P$  = extract from Plato's table, and  $M$  = moisture in %.

$$\text{Extract in sample} = \frac{(\text{Total Extract} - \text{Extract in } 60 \text{ g malt}) \times 100}{40}.$$

## 14.76

## CRUDE FAT OR ETHER EXTRACT.—See 27.25

## 14.77

PROTEIN. See 2.24, 2.25, and 2.26. Multiply results by 6.25.

## 14.78

## ASH.—See 20.5

## 14.79

## CRUDE FIBER.—See 27.30

## HOPS—TENTATIVE

## 14.80

## SAMPLING

(a) *Unpressed Hops*.—Draw equal portions from 5 or 10 different parts of the heap, from surface as well as from different depths, until ca 200 g is obtained. Place sample in tin can having screw or friction-type cover and close tightly.

(b) *Pressed Hops (Bales)*.—Take samples from number of bales representing ca 10% of shipment when under 100 bales, and square root of shipment when over 100 bales. With sharp knife, cut 2 uniform slices of ca 100 g each from opposite points of bale selected. Place the 200 g sample from each bale separately in tin can having screw or friction-type cover and close tightly. Do not use cartons, bags, wooden boxes, glass Mason jars, or wrapping paper as containers for samples. Label samples to show date of sampling; name of company owning the hops at time of sampling; and lot, car, or reference number or letter of identification. If possible, indicate on label origin and year of crop. Store all samples in ice-box or refrigerator pending physical examination and chemical analysis.

## 14.81

## PHYSICAL EXAMINATION

Spread 50-75 g of sample on white paper and examine carefully. In case of a

number of samples of pressed hops, examine each sample separately and combine results for a number of bales or for a full shipment or lot.

(a) *Leaves and Stems (Picking)*.—Ascertain relative number of leaves and stems. Report as very few, few, or many.

If quantitative estimation is required, by means of forceps pick out stems and leaves from 20.00 g of sample, weigh to nearest 0.01 g, and report to first decimal place in percentage. Use remaining portion for seed determination, if required, as directed under (f).

(b) *Color and Luster*.—Determine color and luster on the whole strobiles (cones) and refer findings to predominating character of sample. Report color as greenish-yellow, yellowish-green, pale-green, olive-green, or dark-green. Describe presence of quantities of differently colored cones as: small, medium, or large quantity of . . . cones present, using appropriate color terminology such as brownish, reddish, etc. Report luster as either glossy or dull.

(c) *Size and Condition of Cones*.—Report size according to following classification:

	length (inches)
Large	2½–3
Medium	1½–2
Small	¾–1

Report conditions of cones as unbroken, partly broken, excessively broken.

(d) *Lupulin*.—Break 10 cones into longitudinal halves and examine the lupulin grains thereby exposed as to quantity, color, and condition.

Report quantity as plentiful, fairly plentiful, scarce. Report color as lemon-yellow, orange-yellow, brownish. Report condition as sticky, fairly sticky, not sticky.

(e) *Aroma*.—Rub several cones between hands. Report odor as aromatic, mildly aromatic, abnormal. Use the term “flowery” to describe exceptionally fine aroma. Use designations such as musty, cheesy, etc. to describe abnormal odors.

(f) *Seeds*.—If quantitative estimation is required, dry portion remaining from quantitative determination of leaves and stems or 20.00 g of sample in suitable container in oven for 6 hours, or preferably overnight, at 103–104°. If rapid drying is necessary, place sample in 2 foot squares of muslin or cheesecloth and immerse in quart bowl of methyl alcohol for 1 min. Press out excess alcohol by hand, using rubber gloves for protection. Spread cloth containing hops on a screen to dry in air or over steam radiator.

Rub small portions of dried hops to fine powder between palms of the hands, dropping on a 20-mesh, round sieve. When all sample has thus been comminuted, remove fine particles by shaking sieve thoroly and by blowing. Continue this procedure until portion left on sieve consists mainly of seeds and particles of spindles of the hop cones. Place small portion of this mixture at a time on smooth heavy sheet of paper of convenient size. Holding paper on slight decline, agitate gently and allow seeds to roll from paper into tared dish. Weigh seeds to nearest 0.01 g. Weight  $\times 5$  = percentage of seeds. Report results to first decimal place.

## 14.82

### PREPARATION OF SAMPLE FOR CHEMICAL ANALYSIS

Grind 50–75 g of sample in No. 2 Universal food chopper, using a 12-tooth cutter, or No. 3 Russwin food chopper. Discard first 5 or 10 g. Pass hops evenly and slowly thru the grinder, taking care to avoid choking orifices in order to prevent undue heating of hops. Thoroly mix ground portion into homogeneous mass and store in air-tight container in cool, dark place. (In some cases definite amounts of ground portions from several samples may be mixed together, and analyses run, in duplicate, on mixed portion.)



## 14.83

## MOISTURE

Use one of following procedures, which are listed in order of their accuracy:

- (1) Toluene or tetrachlorethylene distillation method with 10.00 g of sample.
- (2) Vacuum drying at 60° for 3 hours under 22–23" Hg.
- (3) Drying for 1 hour at temp. of 103–104°.

For procedures (2) and (3) use 2.5 g of ground sample in 55 mm weighing bottle or Al dish or 5 g of ground sample in 70 mm dish. (The amount of hops and the dimensions of the dish used are important for accurate results.) Report results in per centage to first decimal place, and state in report which method was used.

## RESINS

## 14.84

## REAGENTS

(a) *Methyl alcohol*.—Absolute C.P.

(b) *Lead acetate soln.*—Dissolve 1 g of Pb acetate, C.P., in absolute methyl alcohol and make to 100 ml after adding 0.1 ml of acetic acid.

## 14.85

## DETERMINATION

*Soft Resins*

Accurately weigh 10 g of well-mixed ground hops into a 500 ml Erlenmeyer or other suitable flask; add by pipet 200 ml of the methyl alcohol, cooled to 20°; stopper with tightly fitting rubber stopper or cork covered with tinfoil; and shake vigorously by machine or hand for 10 min. Filter off ca 100 ml of extract into a 100 ml volumetric flask, cool to 20°, and add sufficient filtrate to bring to mark. Pipet 50 ml of this extract (equivalent to 2.5 g of hops) into 250 ml Squibb separator containing 100 ml of 2% NaCl soln. (Instead of filtration a centrifuge may be used. Cool to 20° and pipet 50 ml of clear, supernatant extract into the separator.)

Extract mixture by shaking 4 successive times with petroleum benzine (b.p. 30–65°), using 45–50 ml for each extraction. Release any pressure developed in separator thru cock. Run off bottom aqueous layer into 200 ml Erlenmeyer flask after each shaking, and run off petroleum benzine layer into plain glass funnel fitted with soft, rapid, filter paper 11 cm in diam. (C.S. and S. No. 589), collecting filtrates in a 250 ml volumetric flask. Return aqueous soln in Erlenmeyer flask to separator, carefully wash out flask with a few ml of petroleum benzine, and transfer this washing to separator. Repeat extraction 3 times. Wash paper and funnel thoroly with petroleum benzine until all traces of resins are dissolved, and make volume of filtrate to 250 ml at 20°. Stopper, and mix thoroly.

Return extracted aqueous methyl alcohol mixture to separator for subsequent determination of hard resins. Transfer 100 ml of petroleum benzine extract (equivalent to 1 g of hops) by means of pipet or buret into tared 150 ml side-arm distillation flask or Soxhlet extraction flask. Evaporate off solvent by distillation on H<sub>2</sub>O or steam bath at 60°, using vacuum and driving off last 5–10 ml by applying suction to flask while it is kept immersed in H<sub>2</sub>O or steam bath, until constant weight is attained (usually after 5 min.). Weigh flask after it has stood near balance for 30 min. In place of either a distillation flask or Soxhlet extraction flask, a large sized Al moisture dish may be used. (In this case, transfer 25 ml of the petroleum benzine extract into the tared dish, evaporate solvent, and dry in a vacuum oven.) Per cent soft resins = weight of resin  $\times$  100.

## 14.86

*Alpha Resins*

(a) *Preliminary titration.*—Transfer 40 ml of the petroleum benzine extract remaining from soft resin determination to small beaker and evaporate off solvent on

water bath. Transfer residue to centrifuge tube of ca 25 ml capacity with small portions of the methyl alcohol to volume of 8 ml, warm to 60°, and add 1.5–2 ml of the Pb acetate soln. Centrifuge liquid and transfer drop of clear, supernatant liquid onto piece of filter paper close beside a drop of 10%  $(\text{NH}_4)_2\text{S}$  or  $\text{Na}_2\text{S}$  soln so that margins of drops interpenetrate. Continue adding the Pb acetate soln (0.5 ml at a time) and centrifuging until drop of supernatant liquid produces a definite brown color with sulfide when spotted on filter paper. Multiply number of ml of Pb acetate soln used by 2.5 and use this quantity for precipitation of alpha resin in main test.

(b) *Precipitation of alpha resin.*—Pipet 100 ml of the petroleum benzine extract (equivalent to 1 g of hops) remaining from soft resin determination into 150 ml side-arm distillation flask or other suitable flask, and remove solvent by distillation on water bath at 60°, using vacuum and driving off last 5–10 ml with suction as directed under 14.85 but at temp. not over 60°. (A vacuum oven may also be used for drying.) Dissolve residue in flask by warming with small quantity of the methyl alcohol and wash into 50 ml beaker until 18–20 ml is obtained. Cover beaker with watch-glass and immerse in water bath at 60° for ca 5 min. Add calculated quantity of the Pb acetate soln (as determined by the preliminary titration) slowly and with constant stirring and digest mixture at 60° for 5 min. Allow beaker to stand at room temp. until precipitate settles and test clear supernatant liquid soln for excess of Pb. If result is negative, heat beaker and contents again to 60° and add 0.5 ml of the Pb acetate soln. Repeat these operations until slight but obvious excess of Pb is indicated by definite brown color in sulfide test. Let soln stand at room temp. for 30 min. and collect precipitate in tared Gooch crucible. Remove all traces of precipitate from beaker to crucible by means of rubber policeman and wash thoroly with the methyl alcohol. Dry crucible and contents to constant weight in oven at 100–101° (1 hour). % alpha resins = weight of Pb salt  $\times 0.631 \times 100$ .

If rapid estimation of alpha resin content is desired, omit preliminary titration, and begin with the 100 ml of petroleum benzine extract. Proceed as directed above and precipitate alpha resin directly by addition of 7 ml of the Pb acetate soln. (This quantity is usually sufficient to precipitate alpha resin from hops having average composition of 16–19% of soft resins.)

In determining the alpha resin content of hops with an exceptionally high or exceptionally low content of soft resins, 1 or 2 ml more or less of the Pb acetate reagent will be required for satisfactory results. Care should be taken to avoid use of excess Pb acetate as this tends to redissolve the precipitated alpha resins. On the other hand, insufficient Pb acetate may cause greater errors than when an excess is used, because insufficient Pb acetate leaves large quantities of alpha resins in soln

#### 14.87

#### *Beta Resins*

% beta resins = % soft resins – % alpha resins.

#### 14.88

#### *Hard or Gamma Resins*

Shake the aqueous methyl alcohol mixture remaining from 14.85 with 4 successive portions of 40 ml each of absolute ether. Use first portion to wash filter paper (previously used to filter petroleum benzine soln of soft resins) and to rinse flask used in separation of aqueous portion from previous petroleum benzine extraction. Collect combined ether extracts in suitable flask and remove ether by distillation. Dry the flask and contents on water or steam bath. Dissolve residue in 5 ml of absolute methyl alcohol and again heat until dry on water or steam bath with constant and rapid twirling of the flask so as to spread extract uniformly on interior of flask. Dry for 1 hour in oven at 105° and weigh when cool.

Dissolve hard resins by means of absolute ether, followed by small quantity of

the methyl alcohol, and discard this mixture, being careful not to lose any NaCl crystals. Dry flask, which contains a slight trace of NaCl, with suction and again weigh. Difference in weight before and after treatment with ether represents hard resins.

% hard resins = difference in weight  $\times 100/2.5$ .

#### 14.89 *Total Resins*

% total resins = % hard resins + % soft resins.

### BREWING SUGARS AND SIRUPS

#### 14.90

##### EXTRACT—TENTATIVE

(a) *Preparation of "10% soln."*—Accurately weigh ca 50 g of well-mixed representative sample, dissolve in warm H<sub>2</sub>O, transfer quantitatively to 500 ml flask, and make to volume at 20°. Mix thoroly.

(b) *Determination.*—By means of suitable pycnometer determine sp. gr. of the soln at 20/20°, as directed under 14.3 or 14.56(d). Obtain corresponding extract from 44.3.

Calculate % extract in original sample from following formula:

$$E = P \times B \times 500 / W, \text{ in which}$$

$E$  = extract of original sample;  $P$  = extract of diluted sample;  $B$  = sp. gr. of diluted sample; and  $W$  = actual weight (ca 50 g) of sample taken. Report to first decimal place.

(c) *Degrees Baumé.*—Obtain degrees Baumé (Modulus 145) equivalent to extract of original sample (b) from 44.3.

#### 14.91

##### NON-EXTRACT (APPARENT WATER)—TENTATIVE

Obtain by subtracting extract of original sample, 14.90(b), from 100.

#### 14.92

##### FERMENTABLE EXTRACT

(a) *Regular Fermentation Method—Official, First Action.*—Ferment 250 ml of the "10% soln" of the sample, 14.90(a), with the equivalent of 5 g of washed, active brewers' compressed yeast at 15–25° for 48 hours or until fermentation is complete. In case of refined sugars and sirups, such as corn sirup, add to soln before fermenting 0.8 g of K<sub>2</sub>HPO<sub>4</sub> crystals, 1 g of NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, and 0.5 g of dried yeast extract, standardized for bacteriological culture media purposes, as nutrients. If such nutrient material needs to be added, redetermine extract of the "10% soln" after adding nutrient material, but before adding yeast. Use fermentation flasks equipped with either H<sub>2</sub>O, Hg, or acid seals and shake flasks several times a day during fermentation. When fermentation is complete, filter soln and determine real extract in filtrate as directed in 14.6(a), after removal of alcohol.

Use following formulas for calculation:

Fermentable extract (on extract basis) =  $(p - n) \times 100$ ; and

Fermentable extract ("as is") =  $(p - n) \times E$ ; where

$P$  = extract of "10% soln" before addition of any nutrients;  $p$  = extract of "10% soln" before fermentation;  $n$  = real extract of "10% soln" after fermentation; and  $E$  = extract of original sample, 14.90(b).

If nutrients have not been used (as in case of malt sirups),  $p = P$ , and will have been determined under 14.90(b), and no re-determination before fermentation is required in such case. Report to first decimal place.

(b) *Rapid Fermentation Method—Tentative.*—Proceed as directed under (a), but use for 250 ml of the “10% soln” of sample, 14.90(a), the equivalent of 40 g of washed, active brewers’ compressed yeast or liquid yeast that has been de-watered by suction on Büchner funnel (more precise results are obtained by washing yeast with the “10% soln” of the sample before final suction filtration). For refined sugars and sirups, use nutrients as directed under (a). Ferment mixture at room temp. (20–23°) and stir continuously with four-blade glass stirrer (ca 2" diam.) at 100–120 r.p.m. until fermentation is complete (4–5 hours). As evaporation can be an important variable, keep agitation and time at minimum. Observe instructions as given under 14.105(b). Finish off fermentation and calculate as directed under (a).

## 14.93

## PROTEIN—TENTATIVE

(a) Transfer 25 ml of the “10% soln,” 14.90(a), to Kjeldahl digestion flask, and proceed as directed in 14.20.

Percent protein ( $N \times 6.25$ ) in original “as is”

$$\text{sample} = \frac{(\text{ml } 0.1 \text{ N H}_2\text{SO}_4 - \text{ml } 0.1 \text{ N NaOH}) \times 0.0014 \times 6.25 \times 500}{25 \times W} \times 100; \text{ where}$$

$W$  = actual weight of sample used in preparing the “10% soln.” Report to second decimal place.

(b) Determine  $N$  as directed in 34.17, and calculate protein, using factor  $N \times 6.25$ .

## 14.94

## DIASTATIC POWER—TENTATIVE

(Malt sirups only)

Transfer 10 ml of the “10% soln,” 14.90(a), to 100 ml volumetric flask and make to volume at 20° with  $\text{H}_2\text{O}$ . Transfer 10 ml of the “1% soln” so prepared to 250 ml volumetric flask, bring to 20°, add 200 ml of buffered starch soln at 20°, 14.62(g), and proceed as directed in 14.63, last four sentences in second paragraph, beginning with words: “Mix soln” and ending “mix thoroly.” Follow Fehling soln modification, 14.63(a).

Calculate diastatic power on “as is” basis according to formula:

$$\text{Degrees Lintner (“as is”)} = \frac{5000 \times B \times 50}{A \times A \times W}; \text{ where}$$

$A$  denotes number of ml of digested starch soln required to reach end point in determination;  $B$  denotes number of ml of digested starch soln required to reach end point in blank; and  $W$  denotes weight of sirup used to prepare the “10% soln.”

Degrees Lintner  $\times 4$  = Maltose Equivalent. Report as Degrees Lintner and Maltose Equivalent to nearest whole number.

## 14.95

## IODINE REACTION FOR UNCONVERTED STARCH—TENTATIVE

Use the “10% soln,” 14.90(a), and proceed as directed in 14.27.

## 14.96

## ACIDITY—TENTATIVE

Transfer 100 ml of the “10% soln,” 14.90(a), to suitable beaker or flask and proceed as directed in 14.10 or 14.11. Calculate and report results as follows:

(a) In terms of ml of 1.0  $N$  NaOH/100 g of sample, “as is”:

$$\text{Acidity} = \frac{\text{ml of } 0.1N \text{ NaOH consumption} \times 500}{10 \times W^*}$$

Report to first decimal.

\*  $W$  represents actual weight (g) of sample used in preparing the “10% soln.”

(b) In terms of "lactic acid" as per cent of sample "as is":

$$\text{Acidity} = \frac{\text{ml of 0.1N NaOH consumption} \times 0.009 \times 500}{W^*}.$$

Report to second decimal.

#### 14.97 H-ION CONCENTRATION (pH)—TENTATIVE

Using the "10% soln," 14.90(a), proceed as directed in 14.12.

#### 14.98 ASH—TENTATIVE

Proceed as directed in 34.9 or 34.10.

#### TOTAL REDUCING SUGARS

#### 14.99 *Munson-Walker General Method—Tentative*

Transfer 50 ml of the "10% soln," 14.90(a), to 250 ml volumetric flask. Clarify, if necessary, with alumina cream or neutral Pb acetate soln only (never basic Pb acetate), and make to volume at 20° with H<sub>2</sub>O. Mix thoroly and either centrifuge or filter until clear. If Pb acetate soln was used for clarification, remove excess Pb with dry Na<sub>2</sub>C<sub>2</sub>O<sub>4</sub>. Filter, and determine reducing sugars on 10 ml aliquot as directed under 34.39, 34.40, 34.51, or 34.59. Calculate results in terms of invert sugar for invert sirups and sugars; dextrose for corn sugars and sirups; and maltose for malt sirups. If character of sample is in doubt, express reducing sugars as dextrose.

#### 14.100 *Lane-Eynon General Volumetric Method—Tentative*

Dilute 50 ml of the soln, clarified as directed under 14.99, to 100 ml and proceed as directed under 34.34, 34.35, 34.50, or 34.58, referring titer to 44.19 or 44.20. Calculate results as directed under 14.99.

#### 14.101 OTHER DETERMINATIONS.—See Chap. 34

#### WORT—TENTATIVE

#### 14.102 PREPARATION OF SAMPLE

Store 1 gallon of wort at 4–7° for 12–15 hours, then filter all but last portion of this sample at 4–7° thru filter paper of the types specified under 14.54(f). If filtrate is not brilliant after first filtration return to filter, but do not use filter-aid. To prevent spoiling, keep sample in refrigerator, and if necessary place in beer bottles and pasteurize. Mix sample well to insure uniformity before removing portion for analysis.

#### 14.103 SPECIFIC GRAVITY

Proceed as directed under 14.3 or 14.56(d). Report to fifth decimal place.

#### 14.104 EXTRACT OF ORIGINAL GRAVITY

From 44.3, ascertain extract corresponding to sp. gr. determined at 20/20°. Report to second decimal place as per cent.

#### 14.105 FERMENTABLE EXTRACT

(a) *Regular method*.—Ferment 250 ml of the wort with equivalent of 5 g of

washed, active brewers' compressed yeast at temp. of 15–25° for 48 hours or until fermentation is complete. Use either H<sub>2</sub>O, Hg, or acid seal to prevent evaporation. Filter soln and determine real extract in filtrate as directed in 14.6(a) after removal of alcohol.

Calculate per cent by weight of fermentable extract as directed under 14.92. Report to second decimal place.

Calculate also real degree of fermentation as directed under 14.8. Report to first decimal place.

(b) *Rapid method.*—(Determines fermentability of worts to ca 14% in 4–5 hours within 0.3–0.1% of attenuation limit.)

To 200 ml of wort in 400–600 ml glass beaker add 32 g of fresh, compressed, washed brewers' lager yeast or liquid yeast that has been dewatered by suction on Büchner funnel (more precise results are obtained by washing yeast with wort to be pitched before final suction filtration). Keep mixture at room temp. (20–23°) and stir continuously with glass stirrer until fermentation is complete (4–5 hours). Filter mixture thru ordinary filter paper, returning first 20 or 30 ml of filtrate to filter. (Filtrate should be clear, but not necessarily brilliant. As evaporation can be an important variable, agitation and time should be kept at minimum. A 4-bladed glass stirrer (ca 2" diam.) operating at 100–120 r.p.m. is satisfactory. Abnormal effects due to autolysis are likely to be indicated by too high a pH of final beer and high calculated original gravity compared to actual original gravity.)

14.106 IODINE REACTION.—See 14.27

14.107 TOTAL ACIDITY.—See 14.10 and 14.11

14.108 H-ION CONCENTRATION (pH).—See 14.12

14.109 COLOR

Prepare sample as directed under 14.102. If prepared wort has developed a haze or sediment that would interfere with determination, clarify by centrifuging, or filtering without use of filter-aid. Indicate such clarification in the report. Determine depth of color of prepared sample in  $\frac{1}{2}$ " cell with Lovibond tintometer as directed under 14.60. Report results in degrees Lovibond,  $\frac{1}{2}$ " cell, Series 52, to first decimal place.

14.110 PROTEIN

Prepare sample as directed under 14.102. If prepared sample shows sediment, mix thoroly to insure perfect distribution of the sediment before removing portion for analysis. To determine protein in the brilliant wort (free from any haze or sediment), reclarify prepared sample by centrifuging or filtering without use of filter-aid. Indicate such clarification in the report.

Pipet 25 ml of prepared sample, measured at 20°, into 800 ml Kjeldahl digestion flask and proceed as directed under 14.20.

14.111 TOTAL REDUCING SUGARS

Prepare sample as directed under 14.102. If appreciable amounts of suspended matter are present in prepared wort, remove by centrifuging or filtering. Transfer 50 ml of sample to 250 ml volumetric flask, make to volume at 20°, and mix thoroly. In general, worts do not require clarification for determination of reducing sugars. If, however, such clarification is necessary, proceed as directed under 14.99. Determine reducing sugars in 10 ml of this soln by the Munson-Walker method as directed

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under 34.59. Or dilute 50 ml of this soln with H<sub>2</sub>O to 100 ml and use the Lane-Eynon method as directed under 34.58. Express results as per cent maltose.

### YEAST

#### *Liquid and Pressed Yeast*

#### TOTAL SOLIDS—TENTATIVE

##### 14.112

##### APPARATUS

- (a) *Sieve*.—Approximately 100-mesh.
- (b) *Moisture oven*.—See 14.49(b).
- (c) *Weighing dish*.—Glass or Al, of at least 65 mm internal diam., with cover, and glass stirring rod of such length that it will fit within covered dish.

##### 14.113

##### REAGENTS

(a) *Alcohol*.—Pure ethyl or methyl alcohol, or alcohol denatured with a completely volatile liquid, such as denatured alcohol Nos. 1, 1-A, 2-B, 3-A, 12-A, 13-A, 23-A.

(b) *Sand*.—Use clean, sharp sand. Wash with H<sub>2</sub>O and dry overnight at 105°. Cool in desiccator. Keep in closed container. Loss of weight of 5 g of this sand when dried for 3 hours at 105° must not exceed 0.005 g.

##### 14.114

##### PREPARATION OF SAMPLE

(a) *Liquid yeast*.—Mix well by stirring.

(b) *Pressed yeast*.—Remove portions from different parts of surface as well as center of cake and collect ca 150 g in 1000 ml beaker. Weigh to nearest 0.1 g. Prepare slurry by adding H<sub>2</sub>O at rate of ca 3 parts of H<sub>2</sub>O/1 part of pressed yeast. Again weigh to nearest 0.1 g. With stirring rod break up yeast portions and stir until completely uniform liquid suspension is obtained.

If lumps or particles of trub are present, pass thru sieve of suitable fineness (ca 100-mesh) the entire bulk sample of liquid yeast, which should amount to not less than 500 ml if the yeast requires screening, or the slurry prepared from ca 150 g of pressed yeast. Make sure that all lumps and particles are broken up and forced thru the sieve. Recover, by scraping, any liquid or solids adhering to sieve and reincorporate them with sieved sample. Mix well by stirring.

##### 14.115

##### DETERMINATION

Place ca 5 g of dry sand in weighing dish. Place dish on pan of balance, together with cover and stirring rod. Obtain tare weight. Transfer to weighing dish ca 10 g of the well-mixed liquid yeast or of the yeast slurry from pressed yeast, cover, and weigh to nearest 0.001 g. Remove cover and add 5 ml of alcohol. By means of stirring rod, mix thoroly. Allow stirring rod to drop into weighing dish. Dry 3 hours (±2 min.) at 105°. Cover, cool in desiccator, and weigh.

For liquid yeast, calculate drying loss of aliquot used to per cent and report as total solids to first decimal place. For pressed yeast, calculate according to following formula:

$$\text{Per cent total solids} = \frac{D \times S \times 100}{W \times P}, \text{ where}$$

$P$  = weight, in g, of pressed yeast used for preparing slurry;  $S$  = total weight, in g,

of the yeast slurry;  $W$  = weight, in g, of slurry aliquot before drying; and  $D$  = weight, in g, of slurry aliquot after drying.

### SPENT GRAINS\*—TENTATIVE

#### 14.116

#### SAMPLING

(a) *Wet Spent Grains*.—Collect by means of scoop numerous small samples at uniform intervals during emptying of tub, so that at end of operation a composite sample of 25–30 lbs. is obtained in clean, dry bucket. From mash filters collect numerous small samples in similar manner at equal time intervals from grain conveyor. Mix thoroly and quarter grains carefully so as to obtain representative sample of 3–4 lbs. Place reduced sample in suitable container having screw or friction-type covers, add few drops of toluene as preservative, and close tightly. Store sample in refrigerator pending analysis.

(b) *Dry Spent Grains*.—See 14.39. Take great care in sampling dry spent grains for analysis, particularly for feed, as experience has shown that it is very difficult to obtain a truly representative sample. Because spent grains are composed of large sized husks and small heavy particles, there is usually a difference in composition at different levels of container. If spent grains are in a sack, empty sack on clean floor and quarter contents down to ca 0.5 lb. for laboratory sample.

If spent grains are in a car, unsacked, it is practically impossible to obtain representative sample, due to segregation at bottom of the fine heavy material, which is higher in protein than the lighter material at top.

Store sample in refrigerator pending laboratory analysis.

On each sample container show date of sampling; name of company owning grains at time of sampling; and brew, lot, car, or reference number or letter for identification.

Before conducting analysis, mix grains thoroly.

#### 14.117

#### PRELIMINARY DRYING (WET SPENT GRAINS)

Weigh out accurately ( $\pm 0.1$  g) ca 1000 g of quartered wet spent grains. Dry in oven at temp. below  $60^\circ$ , or overnight in air by means of fan and heater, until grains appear air-dry. Note accurately ( $\pm 0.1$  g) weight of dried grains and keep in moisture-proof container. Thoroly mix dried sample and grind fine 100 g, as directed under 14.55. Keep ground portion in moisture-proof container.

#### 14.118

#### MOISTURE

(a) *On sample after preliminary drying (when available and soluble extracts are determined)*.—Use 5–10 g of the accurately weighed and ground portions of sample and proceed as directed under 14.51. Calculate percent moisture in dried grains ( $W$ ).

Calculate percent moisture in original wet grains by following formula:

$$\% \text{ moisture in wet grains } (M) = \frac{(W \times D) + 100(G - D)}{G}, \text{ where}$$

$G$  = weight of wet grains before preliminary drying,  $D$  = weight of grains after preliminary drying, and  $W$  = % moisture in grains after preliminary drying.

(b) *On sample in wet condition (when only soluble extract is determined)*.—Use ca 15 g of sample, accurately weighed into 70 mm Al dish, and dry first at temp. below  $60^\circ$  until air-dry. Then dry for 3 additional hours as directed under 14.51.

(c) *On dry spent grains*.—Proceed as directed under (a) and calculate percent moisture.

\* For examination of spent grains for feeding purposes see Chap. 27.



## AVAILABLE EXTRACT

## 14.119

*Wet Spent Grains*

(a) *Apparatus*.—Mash beakers and counter weights, mashing apparatus, filter paper, funnels, flasks, pycnometers, emptying device, and water bath. *See* 14.54.

(b) *Preparation of sample*.—*See* 14.117. *Preparation of finely ground malt*.—*See* 14.56.

(c) *Mashing procedure*.—*See* 14.56(a).

(d) *Cooling and filtration*.—*See* 14.56(c).

(e) *Specific gravity*.—*See* 14.56(d). Ascertain corresponding Plato values from 44.3.

(f) *Calculation*.—Use following formulas:

$$\text{Total extract} = \frac{P \times (800 + W \text{ in } 60 \text{ g malt} + W \text{ in } 40 \text{ g dried grains})}{100 - P}, \text{ where}$$

$P$  = g of extract in 100 g of wort (Plato), and  $W$  = moisture (g).

% available extract in wet grains, dry basis =

$$\frac{(E \text{ in mixture} - E \text{ in } 60 \text{ g malt}) \times 10,000}{40 (100 - M \text{ of dried grains})}, \text{ where}$$

$E$  = extract, and  $M$  = percent moisture.

% available extract in wet grains, as is =

$$\frac{(\text{available } E, \text{ dry basis}) (100 - M \text{ of wet grains})}{100}, \text{ where}$$

$E$  = extract, and  $M$  = percent moisture.

## 14.120

*Dry Spent Grains*

*Preparation of sample*.—Grind finely 100 g of sample and proceed as directed under 14.119. Calculate extract as directed under 14.75.

## SOLUBLE EXTRACT (WET SPENT GRAINS)

## 14.121

*On Sample After Preliminary Drying*

(a) *Mashing procedure*.—"Mash in" in a mash beaker 25 g ( $\pm 0.05$  g) of unground sample with 350 ml of  $H_2O$  at  $71^\circ$ . Place mash beakers in mashing apparatus containing  $H_2O$  previously heated to  $70$ – $71^\circ$  and set stirrers in motion. Hold mash for 60 min. at a mash temp. of  $70^\circ$  ( $\pm 0.5^\circ$ ).

(b) *Cooling and filtering*.—*See* 14.56(c). Make mash to 425 g.

(c) *Specific gravity*.—*See* 14.56(d). Ascertain corresponding Plato values from 44.3.

(d) *Calculation*.—Use following formulas:

% soluble extract in wet grains, dry basis =

$$\frac{P(M \text{ of dried grains} + 1600) \times 100}{(100 - P)(100 - M \text{ of dried grains})};$$

% soluble extract in wet grains, as is =

$$\frac{(E, \text{ dry basis}) (100 - M \text{ of wet grains})}{100}, \text{ where}$$

$P$  = g of extract in 100 g of wort (Plato),  $E$  = ext. and  $M$  = per cent moisture.

## 14.122

*On Sample In Wet Condition*

Using 100 g ( $\pm 0.05$  g) of well-mixed and quartered spent grains and 300 ml of  $H_2O$  at  $71^\circ$ , proceed as directed under 14.121.

*Calculation.*—Use following formulas:

$$\% \text{ soluble extract, as is} = \frac{P(M+350)}{100-P}, \text{ where}$$

$$\% \text{ soluble extract, dry basis} = \frac{E \times 100}{100-M};$$

$P$  = g of extract in 100 g of wort (Plato),  $M$  = per cent moisture content, and  $E$  = soluble extract, "as is".

Report soluble extract on "as is" and "dry basis" in per cent, to first decimal.

## 14.123

*Pressure Method*

By means of hand press, such as a potato masher or fruit press, squeeze out sufficient liquid from spent grains to yield 150–200 ml. Do not use too high pressure, or errors may be introduced. Let coarse particles settle, decant or centrifuge, and filter the liquid, refiltering first portion that comes thru. Determine sp. gr. of liquid as directed under 14.56(d). Calculate corresponding Plato values from 44.3. For rapid estimation in brewery, Plato value of expressed liquid may be ascertained by use of accurate saccharometer.

*Calculation.*—% soluble extract in wet grains, "as is" =  $\frac{P(M)}{100-P}$ ; % soluble extract

in wet grains, dry basis =  $\frac{P(M) \times 100}{(100-P)(100-M)}$ , where

$P$  = g extract in 100 g of wort (Plato), and  $M$  = per cent moisture.

## 14.124

**SOLUBLE EXTRACT (DRY SPENT GRAINS)**

Proceed as directed under 14.121, but use following formula:

$$\% \text{ soluble extract "as is"} = \frac{P(1600+M)}{100-P}, \text{ where}$$

$P$  = g of extract in 100 g of wort (Plato), and  $M$  = %  $H_2O$  in the dry spent grains.

## 15. WINES (1)

### 15.1

#### PHYSICAL EXAMINATION—TENTATIVE

Note and record following: (1) Whether container is "bottle full"; (2) appearance of wine, whether bright or turbid and whether there is any sediment; (3) condition when opened, whether still, gaseous, or carbonated; (4) color and depth of color; (5) odor, whether vinous, foreign or acetous; and (6) taste, whether dry, sweet, vinous, foreign, or acetous.

### 15.2

#### PREPARATION OF SAMPLE—OFFICIAL

Remove any gas in wine by pouring sample back and forth in beakers. Filter wine, regardless of appearance. Determine immediately sp. gr. and those ingredients that are subject to change, such as alcohol, sugars, acids.

### 15.3

#### SPECIFIC GRAVITY—OFFICIAL

Determine sp. gr. at 20/20° by means of a pycnometer as directed under 16.4, or by means of small accurately graduated hydrometer.

### 15.4

#### ALCOHOL—OFFICIAL

(a) *By volume*.—Measure 100 ml of the liquid into 300–500 ml distillation flask, noting temp., and add 50 ml of H<sub>2</sub>O. Attach flask to vertical condenser by means of bent tube, distil almost 100 ml, and make to volume of 100 ml at same temp. (Foaming, which sometimes occurs, especially with young wines, may be prevented by addition of small quantity of tannin.) To determine alcohol in wines that contain an abnormal quantity of acetic acid, add amount of normal NaOH soln necessary to exactly neutralize portion taken (calculated from acidity determination, 15.22) before proceeding with distillation (unnecessary for wines of normal taste and odor). Determine sp. gr. of distillate as directed under 16.4, at room temp. if desired, and obtain corresponding percentage of alcohol by volume from 44.23.

(b) *By weight*.—From 44.25, obtain % alcohol by weight in distillate corresponding to % alcohol by volume, multiply by sp. gr. of distillate, and divide by sp. gr. of sample.

(c) *By immersion refractometer*.—Verify percentages of alcohol (a) and (b) by ascertaining immersion refractometer reading of distillate and obtaining corresponding percentages of alcohol from 44.24.

#### GLYCEROL IN DRY WINES

At no time during any of the evaporations should area of dish exposed to bath be greater in circumference than that covered by liquid in dish (easily done by lowering dish to float in bath).

### 15.5

#### Method I. By Direct Weighing—Official

Evaporate 100 ml of the wine in porcelain dish on water bath maintained at 85–90° to ca 10 ml. Treat residue with ca 5 g of fine sand and 4–5 ml of milk of lime (containing 15 g of CaO/100 ml) for each g of extract present and evaporate almost to dryness. Treat moist residue with 50 ml of alcohol, 90% by volume; remove substance adhering to sides of dish with spatula, and rub whole mass to a paste. Heat mixture on water bath, with constant stirring, to incipient boiling and

decant liquid thru filter into small flask. Wash residue repeatedly by decantation with 10 ml portions of hot 90% alcohol until filtrate amounts to ca 150 ml. Evaporate filtrate to a sirupy consistency in porcelain dish, transfer residue to small, glass-stoppered, graduated cylinder with 20 ml of absolute alcohol, and add 3 portions of 10 ml each of anhydrous ether, shaking thoroly after each addition. Let stand until clear, pour off thru filter, and wash cylinder and filter with mixture of 2 parts of absolute alcohol to 3 parts of anhydrous ether, also pouring wash liquor thru filter. Evaporate filtrate to a sirupy consistency, dry 1 hour at 98–100°, weigh, ignite, and weigh again. Loss on ignition = weight of glycerol.

**15.6**                    *Method II. By Oxidation with Dichromate—Official*

Evaporate 100 ml of wine in porcelain dish on water bath maintained at 85–90° to ca 10 ml. Treat residue with ca 5 g of fine sand and 4–5 ml of milk of lime (containing 15 g of CaO/100 ml). Proceed as directed under 33.76, beginning, “evaporate almost to dryness, with frequent stirring,” except to dilute the soln of glycerol after treatment with  $\text{Ag}_2\text{CO}_3$  and Pb acetate to volume of 100 ml instead of 50 ml. Observe precautions given concerning temp. at which all evaporations are to be made.

**15.7**                    **GLYCEROL IN SWEET WINES—OFFICIAL**

With wines in which the extract exceeds 5 g/100 ml, heat 100 ml to boiling in flask and treat with successive small portions of milk of lime until wine becomes first darker and then lighter in color. Cool, add 200 ml of alcohol, allow the precipitate to subside, filter, and wash with alcohol. Treat the combined filtrate and washings as directed under 15.5 or 15.6.

**15.8**                    **GLYCEROL-ALCOHOL RATIO—OFFICIAL**

Express this ratio as  $X:100$ , in which  $X$  equals percentage weight of glycerol  $\times 100$ ,  $\div$  percentage of alcohol by weight.

**EXTRACT**

**15.9**                    *Method I. From Specific Gravity of Dealcoholized Wine—Official*

Calculate sp. gr. of the dealcoholized wine,  $D$ , by following formula:  $D = S + 1 - A$ ;  $S$  = sp. gr. of sample, 15.3; and  $A$  = sp. gr. of alcoholic distillate, 15.4(a).

From 44.3 ascertain percentage by weight of extract in the dealcoholized wine corresponding to value of  $D$ . This figure  $\times$  value of  $S$  = g of extract/100 ml of wine.

**15.10**                    *Method II. By Evaporation—Official*

(a) *In dry wines, extract content of less than 3 g/100 ml.*—In 75 ml flat-bottomed Pt dish, ca 85 mm in diam., evaporate 50 ml of sample on water bath to sirupy consistency. Heat residue 2–5 hours in drying oven at temp. of boiling  $\text{H}_2\text{O}$ , cool in desiccator, and weigh as soon as dish and contents reach room temp.

(b) *In sweet wines.*—If extract content is 3–6 g/100 ml, treat 25 ml of sample as directed under (a). If extract exceeds 6 g/100 ml, accept result obtained as directed under 15.9, and attempt no gravimetric determination because of inaccurate results obtained by drying levulose at high temp.

**15.11**                    **NON-SUGAR SOLIDS (SUGAR-FREE EXTRACT)—OFFICIAL**

Subtract quantity of reducing sugars before inversion, 15.12, plus sucrose, if present, from extract, 15.9 or 15.10.

## 15.12

## REDUCING SUGARS—OFFICIAL

(a) *Dry wines*.—Place 200 ml of sample in porcelain dish, exactly neutralize with normal NaOH, calculating quantity required from determination of acidity, and evaporate to ca 50 ml. Transfer to 200 ml flask, add sufficient neutral Pb acetate soln, 34.19(d), to clarify, dilute to mark with H<sub>2</sub>O, shake, and pass thru folded filter. Remove the Pb with dry K oxalate and determine reducing sugars as directed under 34.39.

(b) *Sweet wines*.—Approximate sugar content by subtracting 2 from extract, 15.9 or 15.10, and use such quantity of sample that aliquot taken for Cu reduction contains not over 240 mg of invert sugar. Proceed as directed under (a).

## SUCROSE

## 15.13

*I. By Reducing Sugars Before and After Inversion—Official*

Proceed as directed under 34.30, using method given under 34.39, for the determination of reducing sugars.

## 15.14

*II. By Polarization—Official*

Polarize before and after inversion in 200 mm tube, as directed under 34.23 or 34.24, a portion of filtrate obtained under 15.12. In calculating percentage of sucrose do not fail to take into consideration relation of weight of sample contained in 100 ml to normal weight for instrument.

## 15.15

## COMMERCIAL GLUCOSE—OFFICIAL

Polarize a portion of filtrate obtained under 15.12, after inversion in 200 mm jacketed tube at 87°, as directed under 34.32. In calculating percentage of glucose do not fail to take into consideration relation of weight of sample contained in 100 ml to normal weight for instrument.

## 15.16

## ASH—OFFICIAL

Proceed as directed under 34.9 or 34.10, using residue from 50 ml of wine. Char carefully (decrepitation), and do not exceed 550° during ashing.

## 15.17

## ALKALINITY OF WATER-SOLUBLE ASH—OFFICIAL

Extract ash obtained as directed under 15.16 with successive small portions of hot H<sub>2</sub>O until filtrate amounts to ca 60 ml and proceed as directed under 34.14. Express alkalinity in terms of ml of 0.1 *N* acid required to neutralize the water-soluble ash from 100 ml of the wine.

## 15.18

## ALKALINITY OF WATER-INSOLUBLE ASH—OFFICIAL

Ignite filter and residue from 15.17 in the Pt dish in which the wine was ashed and proceed as directed under 34.15. Express alkalinity in terms of ml of 0.1 *N* acid required to neutralize the water-insoluble ash from 100 ml of the wine.

## 15.19

## PHOSPHORIC ACID—OFFICIAL

Dissolve the ash, 15.16, in 50 ml of boiling HNO<sub>3</sub> (1+9), filter, wash paper, and determine P<sub>2</sub>O<sub>5</sub> in combined filtrate and washings as directed under 2.9 or 2.12. If ash ignites without difficulty, no free H<sub>3</sub>PO<sub>4</sub> need be suspected. If any free acid is present, ash remains black even after repeated leaching. In latter case, add Ca acetate or mixture containing 3 parts of Na<sub>2</sub>CO<sub>3</sub> and 1 part of NaNO<sub>3</sub> to avoid loss of P<sub>2</sub>O<sub>5</sub> before attempting to ash.

## 15.20

## SULFURIC ACID—OFFICIAL

Acidify 50 ml of sample with small excess of HCl, and precipitate  $\text{H}_2\text{SO}_4$  with 10%  $\text{BaCl}_2$  soln. Treat the  $\text{BaSO}_4$  as directed under 12.34 except to allow precipitate to stand for at least 6 hours. Report as  $\text{SO}_3$ , using factor 0.3430.

## 15.21

## CHLORIDES—OFFICIAL

To 100 ml of dry wine or 50 ml of sweet wine, add sufficient  $\text{Na}_2\text{CO}_3$  to make distinctly alkaline. Evaporate to dryness, ignite at heat not above  $550^\circ$ , cool, extract residue with hot  $\text{H}_2\text{O}$ , acidify  $\text{H}_2\text{O}$  extract with  $\text{HNO}_3$  (1+4), and determine chlorides as directed under 12.42 or 12.44.

## 15.22

## ACIDITY—OFFICIAL

(a) *Phenolphthalein*.—Add ca 1 ml of phenolphthalein indicator to ca 250 ml of recently boiled  $\text{H}_2\text{O}$  in large porcelain dish. Neutralize with 0.1 *N* NaOH, 7.6(a). Quantity of sample to be used depends on depth of color of wine; it is generally 5 ml for deeply colored red wine and 20 ml for white wine. Titrate rapidly to distinct pink. Heat portion of wine to be titrated to incipient boiling to remove  $\text{CO}_2$  (all wines, still or gaseous) and transfer to dish with portion of the neutralized  $\text{H}_2\text{O}$ .

(b) *Azolitmin*.—Measure 20 ml of wine into 250 ml beaker, heat rapidly to incipient boiling, and immediately titrate with 0.1 *N* NaOH. Determine end point with neutral 0.05% azolitmin soln as outside indicator. Place indicator in cavities of spot plate and spot wine into azolitmin soln. End point is reached when color of indicator remains unchanged by addition to wine of few drops of 0.1 *N* NaOH.

(c) *Phenolphthalein powder (artificially colored wines)*.—Into cavities of a spot plate place mixture of one part phenolphthalein and 100 parts dry powdered  $\text{K}_2\text{SO}_4$  and spot wine into powder. (End of titration is indicated when powder acquires pink tint. Powder should not be too fine. Addition of neutral alcohol to wine will facilitate flow of wine into powder.) Tilt spot plate and allow wine to flow into powder from tip of heavy stirring rod.

Express results in terms of tartaric acid. 1 ml of 0.1 *N* NaOH = 0.0075 g of tartaric acid.

## TOTAL VOLATILE ACIDITY

## 15.23

## Method I—Official

Heat rapidly to incipient boiling 50 ml of wine in 500 ml distillation flask, connected with condenser, and pass steam thru until 15 ml of distillate requires only 2 drops of 0.1 *N* NaOH for neutralization. Boil  $\text{H}_2\text{O}$  used to generate the steam several minutes before connecting steam generator with distillation flask in order to expel  $\text{CO}_2$ . Titrate rapidly with 0.1 *N* NaOH, using phenolphthalein indicator. (Color should remain ca 10 seconds.) Express results as acetic acid. 1 ml of 0.1 *N* NaOH = 0.0060 g of acetic acid.

## 15.24

## Method II (2)—Official

Introduce 10 ml of wine, freed from excess  $\text{CO}_2$  by pouring back and forth between large beakers, into inner tube of modified Hortvet type of distillation apparatus (Fig. 20). (Preferably use sufficiently large inner Sellier tube (ca  $1\frac{1}{2} \times 8''$ ) and large distillation trap.) Place 150 ml of recently boiled hot  $\text{H}_2\text{O}$  in outer flask. Connect with slanting or vertical straight tube condenser and distil, by heating outer flask, into 300 ml Erlenmeyer flask (marked at ca 80 ml capacity), until 80 ml of distillate has been obtained. If wine is new or is charged with  $\text{CO}_2$ , bring distillate to

boiling, boil 30 seconds, and titrate hot with 0.1 *N* NaOH, using phenolphthalein indicator. As an alternative, adjust H<sub>2</sub>O flow thru condenser so that condensate is received hot. Distil at such a rate as to obtain the 80 ml in ca 10 min. For wines with abnormally high acetic acid content, continue distillation and titrate each succeeding 10 ml of distillate until not more than 1 drop of standard alkali is required to reach neutral point. If wine is free of CO<sub>2</sub>, or has been previously freed from CO<sub>2</sub> by heating to incipient boiling and cooling or by shaking thoroly in vacuo in a flask connected to water aspirator, distillate may be titrated cold. Use 10 ml buret graduated in 1/20 or 1/50 ml.

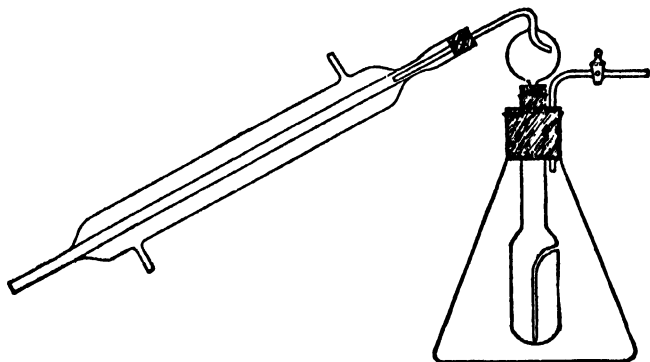


FIG. 20.—APPARATUS FOR DETERMINATION OF VOLATILE ACIDITY

#### VOLATILE ACIDITY—EXCLUSIVE OF SO<sub>2</sub>

##### 15.25

##### *Method I—Tentative*

Immediately after neutralization, if necessary, cool distillate, 15.23 or 15.24, to room temp. by plunging in ice bath; add rapidly to 100 ml of distillate from graduated cylinder 5 ml of H<sub>2</sub>SO<sub>4</sub> (1+3) and several ml of starch indicator; and titrate with 0.1 *N* I, using 10 ml buret. Subtract ml of 0.1 *N* I used from ml of 0.1 *N* NaOH used, 15.23 or 15.24, and express results as g of acetic acid/100 ml of wine.

##### 15.26

##### *Method II—Tentative*

Pipet 50 ml of wine into 100 ml volumetric flask. If white, add 2-3 drops of phenolphthalein indicator and neutralize to decided pink by clear saturated Ba(OH)<sub>2</sub> soln; if red, add sufficient Ba(OH)<sub>2</sub> soln to bring mixture to ca pH 8, using phenolphthalein as external indicator. Allow mixture to stand 30 min. and maintain at phenolphthalein end point by addition of more Ba(OH)<sub>2</sub>, if necessary. Make up to 100 ml, mix, and filter rapidly thru fluted, rapidly filtering paper (such as Whatman No. 2). Pipet 20 ml of filtrate into inner Sellier tube of Hortvet type apparatus, using larger type tube, and add 1 ml of H<sub>2</sub>SO<sub>4</sub> (1+3); place 150 ml of recently boiled hot H<sub>2</sub>O in outer flask; and distil 100 ml. Titrate with 0.1 *N* NaOH, using phenolphthalein indicator.

##### 15.27

##### FIXED ACIDITY—OFFICIAL

Calculate fixed acidity as tartaric acid by multiplying total volatile acidity by 1.25 and subtracting product from total acidity.

## 15.28

## TOTAL TARTARIC ACID (3)—OFFICIAL

Neutralize 100 ml of sample with  $N$  NaOH, calculating from acidity, 15.22, number of ml of  $N$  alkali necessary. If more than 10 ml of alkali is added, evaporate to ca 100 ml. Add to neutralized soln 0.075 g of tartaric acid for each ml of  $N$  alkali added. It is essential that the tartaric acid be pure; if necessary recrystallize. After tartaric acid has dissolved, add 2 ml of acetic acid and 15 g of KCl. After KCl has dissolved, add 15 ml of alcohol, stir vigorously until K bitartrate begins to precipitate, and let stand in icebox at 15–18° at least 15 hours. Decant liquid onto Gooch crucible prepared with very thin film of asbestos, or onto filter paper in Büchner funnel. Wash precipitate from beaker with filtrate (keep cold) and finally rinse beaker and filter 3 times with few ml of mixture of 15 g of KCl, 20 ml of alcohol, and 100 ml of  $H_2O$ , using not more than 20 ml of wash soln in all. Transfer asbestos or paper and precipitate to beaker in which precipitation was made; wash Gooch crucible or Büchner funnel with hot  $H_2O$ , using ca 50 ml in all; heat to boiling; and titrate hot soln with 0.1  $N$  NaOH, using phenolphthalein indicator. Increase number of ml of 0.1  $N$  alkali required by 1.5 ml to allow for solubility of precipitate. Under these conditions 1 ml of 0.1  $N$  alkali = 0.015 g of tartaric acid. To obtain grams of total tartaric acid/100 ml of wine, subtract quantity of tartaric acid added from this result.

## 15.29

## FREE TARTARIC ACID AND CREAM OF TARTAR (4)—OFFICIAL

Calculate in following manner:  $A$  = total tartaric acid, 15.28, divided by 0.015;  $B$  = total alkalinity of ash ( $C + D$ );  $C$  = alkalinity of water-soluble ash, 15.17; and  $D$  = alkalinity of water-insoluble ash, 15.18.

(1) If  $A$  is greater than  $B$ , cream of tartar =  $0.0188 \times C$ , and free tartaric acid =  $0.015 \times (A - B)$ ;

(2) If  $A$  equals  $B$  or is smaller than  $B$  but greater than  $C$ , cream of tartar =  $0.0188 \times C$ , and free tartaric acid = 0; and

(3) If  $A$  is smaller than  $C$ , cream of tartar =  $0.0188 \times A$ , and free tartaric acid = 0.

## 15.30

## CITRIC AND MALIC ACIDS—TENTATIVE

For citric and malic acids occurring in normal wines in small quantities only, use 100 ml of sample and evaporate to 45 ml. After saponification proceed as directed under 26.37, 26.40, or 26.43.

## 15.31

## LACTIC ACID (5)—OFFICIAL

Transfer 25 ml of wine to 250 ml volumetric flask, add ca 25 ml of  $H_2O$  and 100 ml of alcohol, and shake vigorously. Make to mark with alcohol and filter thru folded filter paper. Transfer 200 ml of filtrate to 400 ml beaker and evaporate to ca 25 ml. Add 50 ml of  $H_2O$  and again evaporate to 25 ml. Transfer material to continuous extractor with 25 ml of  $H_2O$  and proceed as directed under 22.13.

## TANNIN AND COLORING MATTER—OFFICIAL

## 15.32

## REAGENTS

(a) *Oxalic acid soln.*—0.1  $N$ . 1 ml = 0.00416 g of tannin.

(b) *Standard potassium permanganate soln.*—Dissolve 1.333 g of  $KMnO_4$  in 1 liter of  $H_2O$  and standardize soln against (a).

(c) *Indigo soln.*—Dissolve 6 g of Na indigotin disulfonate in 500 ml of  $H_2O$  by heating; cool, add 50 ml of  $H_2SO_4$ , make up to 1 liter, and filter.

(d) *Purified boneblack.*—Boil 100 g of finely powdered boneblack with successive



portions of HCl (1+3), filter, and wash with boiling H<sub>2</sub>O until free from chlorides. Keep covered with H<sub>2</sub>O.

## 15.33

## DETERMINATION (6)

Dealcoholize 100 ml of the wine by evaporation and dilute with H<sub>2</sub>O to original volume. Transfer 10 ml to 2 liter porcelain dish and add ca 1 liter of H<sub>2</sub>O and exactly 20 ml of the indigo soln. Add the standard KMnO<sub>4</sub> soln, 1 ml at a time, until blue color changes to green; then add few drops at a time until color becomes golden yellow. Designate number of ml of KMnO<sub>4</sub> soln as "a."

Treat 10 ml of the prepared dealcoholized wine 15 min. with boneblack, filter, and wash thoroly with H<sub>2</sub>O. Add 1 liter of H<sub>2</sub>O and 20 ml of the indigo soln and titrate with KMnO<sub>4</sub>, as directed above. Designate number of ml of KMnO<sub>4</sub> used as "b."

Then  $a - b = c$ , number of ml of the KMnO<sub>4</sub> soln required for oxidation of tannin and coloring matter in 10 ml of wine.

## 15.34

## CRUDE PROTEIN—OFFICIAL

Determine N in 50 ml of the wine as directed under 2.24, 2.25, or 2.26, and multiply result by 6.25.

## 15.35

## PENTOSANS—OFFICIAL

(Applicable to dry wines only)

Proceed as directed under 27.39, except to use 100 ml of the wine and 43 ml of HCl in beginning distillation.

## 15.36

## NITRATES—TENTATIVE

(a) *White wine*.—Treat a few drops of wine in porcelain dish with 2–3 ml of H<sub>2</sub>SO<sub>4</sub> that contains ca 0.1 g of diphenylamine (?) per 100 ml. The deep blue color formed in presence of nitrates appears so quickly that it is not obscured, even in sweet wine, by blackening produced by action of H<sub>2</sub>SO<sub>4</sub> on the sugar.

(b) *Red wine*.—Clarify with basic Pb acetate, filter, remove Pb from filtrate with Na<sub>2</sub>SO<sub>4</sub>, filter again, and treat a few drops of this filtrate as directed under (a).

## 15.37

## COLORING MATTERS—TENTATIVE.—See Chap. 21.

## CARAMEL

## 15.38

*Milos Test (8)—Official*

To 25 ml of sample in an Erlenmeyer flask add 50 ml of alcohol and 50 ml of ether, stopper, shake well, and allow to stand. (When necessary to use a larger sample, increase quantities of alcohol and ether proportionately.) If 2 immiscible layers form after soln has stood for some time add more alcohol (25 ml is usually sufficient), shake well, and allow to stand. In presence of appreciable quantities of caramel, amorphous precipitate will form on bottom of flask within a few hours. If no precipitate forms after a few hours, allow flask to stand overnight.

Filter soln thru Büchner funnel, using filter paper. Wash residue remaining in flask and on filter paper 3 times with hot 85% alcohol containing 0.5% HCl, using 10–15 ml portions until filtrate (in case of red wine) no longer shows presence of red or pinkish color. Then wash twice with hot 85% alcohol.

Place filter paper in flask in which precipitation originally took place, add 10 ml of H<sub>2</sub>O, and heat gently until residue dissolves. If precipitate does not dissolve readily, make aqueous soln alkaline with 2% KOH soln, adding 1 or 2 drops and heating

gently until precipitate dissolves. Cool, filter, and test for caramel with following reagents:

To 2 ml of soln add 0.5 ml of 10% NaOH soln and boil (caramel darkens slightly). Cool, and add 10 ml of Marsh reagent, 16.37. Caramel does not dissolve in Marsh reagent.

To 2 ml of soln add a few drops of HCl (caramel lightens slightly), add 10 ml of Marsh reagent, 16.37, and shake well (caramel does not dissolve in Marsh reagent). Change of color to pink or red on addition of the HCl indicates presence of wine colors.

To 2 ml of clear filtered caramel soln in 10 ml glass-stoppered graduated cylinder add 3 or 4 times the volume of paraldehyde and just sufficient absolute alcohol to form homogeneous and clear soln. Formation of brownish precipitate indicates caramel. Samples containing small amounts of caramel may need to stand overnight.

To 2 ml of soln add an equal volume of freshly prepared (at time of test) reagent consisting of phenylhydrazine hydrochloride, 2 parts; Na acetate, 3 parts; H<sub>2</sub>O, 20 parts. Add 2 parts of saturated NaHSO<sub>3</sub> soln as stabilizer. Dark brown precipitate forms in presence of caramel. Run a blank with reagent and H<sub>2</sub>O at same time samples are being run.

Dilute ca 2 ml of soln with ca equal volume of alcohol, and test with cyclohexanol reagent, 16.40, as directed for the Marsh reagent.

#### 15.39

#### *Confirmatory Test—Tentative*

Place 10–20 ml of wine in small carefully balanced centrifuge tube (Babcock test bottle); neutralize with necessary quantity of 0.1 *N* NaOH calculated from acidity determination; add 2 ml of 5% ZnCl<sub>2</sub> soln and 2 ml of 2% KOH soln, stir well, and centrifuge 5 min. or more. Decant liquid, add 25 ml of boiling H<sub>2</sub>O, mix, centrifuge, and decant. Repeat operation until aqueous liquid is colorless. Caramel forms compound or addition product with the Zn(OH)<sub>2</sub>, which is insoluble in boiling H<sub>2</sub>O and remains after a number of washings. To this well-washed residue (usually 2 or 3 ml) add 25 ml of 85% alcohol containing 0.5% HCl. Shake well and again centrifuge 5–10 min. If present, caramel forms tight layer on bottom of Babcock bottle and may be washed with any number of alcohol-acid washings, altho one or two washings are usually enough. In absence of caramel usually no residue remains and there is no need for paraldehyde or phenylhydrazine hydrochloride confirmatory tests. Add 6–10 ml of boiling H<sub>2</sub>O to any residue and shake to dissolve the caramel. If there are a few insoluble particles, add 1 or 2 drops of 2% KOH soln with slight warming to dissolve.

Divide into 3 portions. To one add 3–5 volumes of paraldehyde in 10 ml glass-stoppered cylinder and just sufficient absolute alcohol to form clear homogeneous soln (carefully avoid excess). Caramel will be indicated by formation of brownish precipitate on standing.

If the soln to be tested with paraldehyde is cloudy, filter into the small 10 ml cylinder where the paraldehyde and absolute alcohol are to be added.

To another portion of the caramel soln add an equal volume of freshly prepared reagent consisting of phenylhydrazine hydrochloride, 2 parts; Na acetate, 3 parts; H<sub>2</sub>O, 20 parts. Add the phenylhydrazine hydrochloride and Na acetate to the H<sub>2</sub>O and stir until all are completely dissolved; add 2 parts of saturated NaHSO<sub>3</sub> soln as a stabilizer. (Dark brown precipitate forms in presence of caramel.) Run blank with reagent and H<sub>2</sub>O at same time samples are run.

Dilute the third portion with  $\frac{1}{2}$  its volume of alcohol and apply the Marsh test, 16.38, and cyclohexanol test, 16.40, to portions of the mixture.

## 15.40

## SULFUROUS ACID (9)—OFFICIAL

Proceed as directed under 32.32, using 100–300 ml of wine containing not more than 40 mg of  $\text{SO}_2$ . Sweep out flask thoroly with  $\text{CO}_2$  or  $\text{N}_2$ , stop flow of gas, and introduce sample into flask thru dropping funnel. Add sufficient S-free  $\text{H}_2\text{O}$  to make total volume of 300 ml, and then add 20 ml of  $\text{HCl}$ . Allow mixture to stand few minutes until fumes have settled. Adjust burner so that vapors rise no higher than one-tenth the length of water jacket of condenser and boil sample 90 min. Adjust flow of gas so that slow but steady stream passes thru receiver during distillation. Complete analysis as directed in 32.32. Report results as mg of  $\text{SO}_2$ /liter. ( $\text{SO}_2$  in wine is unstable. For this reason the wine should be given no preparatory degassing treatment and exposure to air should be minimized prior to the determination.)

## 15.41

## PRESERVATIVES—OFFICIAL

Proceed as directed under Chap. 32.

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## 16. DISTILLED LIQUORS

### SPIRITS

#### 16.1

##### PHYSICAL EXAMINATION—TENTATIVE

Note and record following: (1) color and depth of color; (2) odor—whisky, brandy, rum, etc., or foreign; (3) taste—whisky, brandy, rum, etc., or foreign.

##### SPECIFIC GRAVITY (APPARENT)—OFFICIAL

#### 16.2

##### APPARATUS

(a) *Constant temperature water bath.*

(b) *Pycnometers.*—100 ml (Fig. 21), 50 ml (Fig. 22).

#### 16.3

##### CALIBRATION

Clean pycnometer by use of a chromate- $\text{H}_2\text{SO}_4$  soln and rinse thoroly with  $\text{HO}_2$ .

Fill pycnometer above graduation mark with recently boiled  $\text{H}_2\text{O}$ , insert stopper, and immerse in constant temp. water bath maintained at desired temp. so that water level of bath is above graduation mark on pycnometer. After 30 min., remove stopper and by means of finely drawn out capillary tube adjust until bottom of meniscus is tangent to graduation mark. A hand lens aids in making adjustment.

With small roll of filter paper, dry inside neck of pycnometer above meniscus. Stopper, and immerse in  $\text{H}_2\text{O}$  at room temp. for 15 min. Remove pycnometer, dry with clean lintless cloth, let stand 15 min., and weigh. Empty, rinse several times with alcohol followed by ether, or several times with acetone, and dry thoroly in air with suction. Allow empty flask to come to room temp., stopper, and weigh. Ascertain weight in air of contained  $\text{H}_2\text{O}$  by subtracting weight of empty pycnometer from weight when filled.

#### 16.4

##### DETERMINATION

Obtain weight of sample as directed in 16.3.

Specific gravity in air =  $S/W$ , where  $S$  = weight of sample, and  $W$  = weight of  $\text{H}_2\text{O}$ .

##### ALCOHOL BY VOLUME—OFFICIAL

#### 16.5

##### APPARATUS

(a) *Distillation apparatus.*—500 ml flask.

(b) *Liebig condenser.*—With jacket at least 400 mm, inner tube inside diam. of 9 mm  $\pm$  1 mm, assembled vertically, with adapter attached with rubber tubing.

(c) *Connecting bulb.*—Iowa State type is convenient.

(d) *Connections.*—Use live rubber or ground-glass joints.

(e) *Electric- or gas-operated heating unit.*

#### 16.6

##### DETERMINATION

(a) *For samples containing 60% or less alcohol by volume.*—Calibrate as directed in 16.3, 100 ml pycnometer (Fig. 21) at one of the temperatures specified in 44.23. Fill the clean dry pycnometer with sample and adjust to volume at temp. of calibration as directed in 16.3.

Transfer contents of pycnometer to distilling flask, which has just been rinsed with cold  $H_2O$  and to which has been added a few glass beads or equivalent. Rinse pycnometer three times, using total of 25 ml (40 ml for cordials) of cold  $H_2O$ , and add rinse  $H_2O$  to flask. Place the wet pycnometer so that adapter extends just into bulb. Surround pycnometer with ice or ice water. Complete connections and pass thru water jacket a rapid stream of  $H_2O$  maintained at not over  $25^\circ$  at outlet. Distil ca 96 ml at uniform rate in not less than 30 nor more than 60 min., using longer periods of time for higher percentages of alcohol. Remove and stopper pycnometer, mix distillate by swirling, and wash down with  $H_2O$  any drops that may be above graduation mark. Immerse in constant temp. bath at calibration temp. and after 30 min. carefully make up to volume, with aid of capillary tube, by adding  $H_2O$  previously boiled

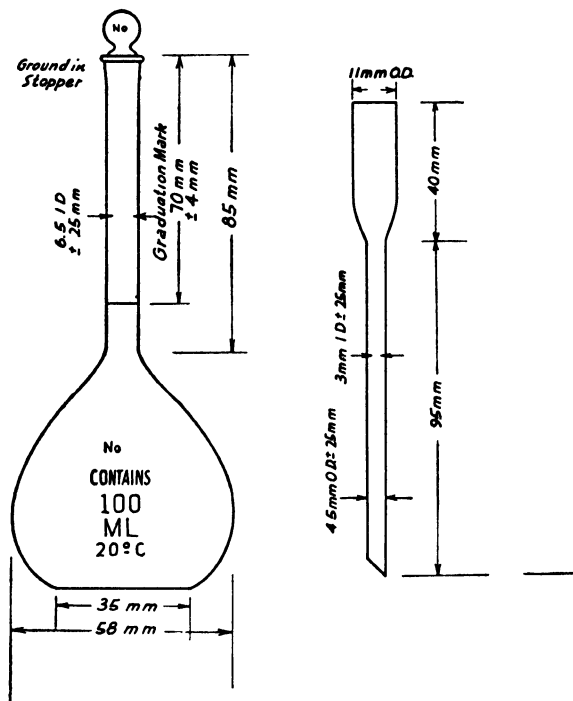


FIG. 21.—100 ML PYCNOMETER

and cooled to same temp. Determine sp. gr. of distillate as directed under 16.4. Obtain corresponding percentage of alcohol by volume from 44.23. (This result is per cent alcohol by volume at  $15.56^\circ$  ( $60^\circ F$ ).)

(b) *For samples containing more than 80% alcohol by volume.*—Proceed as directed under (a) with following changes: Calibrate 100 ml and 50 ml pycnometers (Figs. 21 and 22) at  $15.56^\circ$ , fill 50 ml pycnometer with sample, and adjust to volume at  $15.56^\circ$ . Add 50 ml of cold  $H_2O$  to distilling flask before transfer of sample and collect distillate in 100 ml pycnometer. Obtain sp. gr. of distillate, and from table, 44.23, obtain per cent alcohol by volume in distillate. Compute as follows:

$$\text{Per cent alcohol by vol. in sample at } 15.56^\circ = \frac{D \times W}{W'}, \text{ where } D = \text{per cent alcoho}$$

by vol. in distillate at 15.56°;  $W$  = weight of  $H_2O$  at 15.56° in 100 ml pycnometer; and  $W'$  = weight of  $H_2O$  at 15.56° in 50 ml pycnometer.

## 16.7

## ALCOHOL BY WEIGHT—OFFICIAL

Weigh accurately 40–50 g sample, using clean dry 50 ml pycnometer (Fig. 22) or other closed vessel. (When alcohol is 60% or less by volume, the 100 ml of sample of 16.6 (a) may be weighed and used.) Transfer to 500 ml distillation flask containing 50 ml of  $H_2O$  and a few clean glass beads or equivalent. Rinse pycnometer three times, bringing contents of distillation flask to ca 125 ml. Distil and determine per cent alcohol by volume in distillate as directed under 16.6 (a). Ascertain corresponding per cent alcohol by weight in distillate from table, 44.25. Multiply result by weight of distillate and divide by weight of sample.

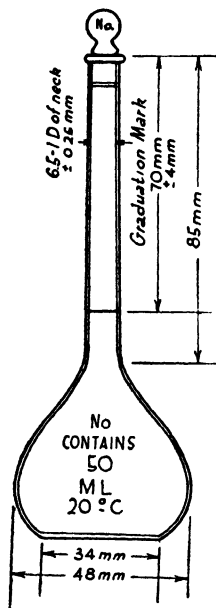


FIG. 22.—50 ML PYCNOMETER

## 16.8

## EXTRACT—TENTATIVE

Weigh, or measure at 20°, 25–100 ml of sample, evaporate to dryness on steam bath, transfer to water oven, and dry at temp. of boiling  $H_2O$  30 min.

## 16.9

## ASH—OFFICIAL

Proceed as directed under 34.9 or 34.10, using residue from 16.8.

## 16.10

## TOTAL ACIDS—OFFICIAL, FIRST ACTION

Neutralize ca 250 ml of boiled  $H_2O$  in porcelain evaporating dish (7½" dish is convenient). Add 25 ml of sample and titrate with 0.1 N NaOH, using ca 2 ml of phenolphthalein indicator soln, 7.6(a).

## 16.11

## FIXED ACIDS—TENTATIVE

Evaporate 25–50 ml of sample to dryness in Pt dish on steam bath and dry 30 min. in oven at 100°. Dissolve, and transfer residue with several portions of neutral alcohol of ca same proof as sample, using 25–50 ml in all, to porcelain dish containing ca 250 ml of neutralized boiled H<sub>2</sub>O. Titrate with 0.1 N NaOH using 10 ml buret graduated in 0.05 ml and same amount of indicator as under 16.10.

## 16.12

## VOLATILE ACIDS—OFFICIAL, FIRST ACTION

Volatile acids = total acids, 16.10,—fixed acids, 16.11.

## 16.13

## ESTERS—TENTATIVE

Measure 100–200 ml of sample into distillation flask, add 12.5–25 ml of H<sub>2</sub>O, and distil slowly 100–200 ml, depending upon amount of sample taken, using mercury valve to prevent loss of alcohol. Exactly neutralize free acid in 50 ml of distillate with 0.1 N NaOH, then add a quantity of 0.1 N NaOH ca 1.5 times that required to saponify the esters. Connect flask with air-cooled condenser ca 2 ft. long, heat 2 hours on steam bath, allow to cool, and titrate excess alkali. Calculate ml of 0.1 N NaOH used in saponification of the esters as ethyl acetate. 1 ml of 0.1 N NaOH = 0.0088 g of ethyl acetate. Run blank, using H<sub>2</sub>O in place of distillate, and make any necessary correction.

## ALDEHYDES

*Colorimetric Method—Official*

## 16.14

## REAGENTS

(a) *Aldehyde-free alcohol*.—Redistil alcohol over NaOH or KOH, add 2–3 g per liter of metaphenylenediamine hydrochloride, digest at ordinary temp. several days (or under reflux condenser on steam bath several hours), and distil slowly, rejecting first 100 ml and last 200 ml of distillate.

(b) *Sulfite-fuchsin soln*.—Dissolve 0.50 g of pure fuchsin in 500 ml of H<sub>2</sub>O, add 5 g of SO<sub>2</sub> dissolved in H<sub>2</sub>O, make up to 1 liter, and allow to stand until colorless. As this soln decomposes rapidly, prepare it in small quantities and keep at low temp.

(c) *Standard acetaldehyde soln*.—Prepare as follows (1): Grind aldehyde ammonia in mortar with anhydrous ether and decant the ether. Repeat this operation several times and dry purified salt in current of air and then in vacuo over H<sub>2</sub>SO<sub>4</sub>. Dissolve 1.386 g of this purified aldehyde ammonia in 50 ml of alcohol, add 22.7 ml of N alcoholic H<sub>2</sub>SO<sub>4</sub>, make up to 100 ml with alcohol, and add 0.8 ml of alcohol for the volume of the (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> precipitate. Allow mixture to stand overnight, and filter. This soln contains 1 g of acetaldehyde in 100 ml and will retain its strength.

Dilute 2 ml of above soln to 100 ml with alcohol (50% by volume). 1 ml = 0.0002 g of acetaldehyde. Prepare soln every day or so.

## 16.15

## DETERMINATION

Determine aldehyde in prepared distillate, 16.13. Dilute 5–10 ml of distillate to 50 ml with aldehyde-free alcohol, 50% by volume; add 25 ml of the sulfite-fuchsin soln, and allow to stand 15 min. at 15°. Solns and reagents should be at 15° when they are mixed. Prepare standards of known strength and blanks in same way. Comparison standards found most convenient for use contain 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg of acetaldehyde.

*Volumetric Method (2)—Official, First Action*

## 16.16

## REAGENTS

(a) *Sodium thiosulfate soln.*—0.05 *N.* Standardize against 0.05 *N*  $K_2Cr_2O_7$  as follows: Place 20 ml of 0.05 *N*  $K_2Cr_2O_7$  in glass-stoppered flask and add 5 ml of 15% KI soln. Add 2.5 ml of HCl and dilute with 100 ml of  $CO_2$ -free  $H_2O$ , then immediately titrate the liberated I with the  $Na_2S_2O_3$  soln until yellow color has almost disappeared; add 1–2 ml of starch indicator, 2.58 (d), and continue, with constant shaking, addition of  $Na_2S_2O_3$  soln until blue color just disappears.

(b) *Iodine soln.*—0.05 *N.* Standardize this soln against the  $Na_2S_2O_3$  soln.

(c) *Sodium bisulfite soln.*—Approximately 0.05 *N.* (This soln will not deteriorate so fast if it contains 5–10% of alcohol; it should not be used after 2–3 days.)

## 16.17

## DETERMINATION

Pipet 50 ml of sample into Erlenmeyer flask and add 10 ml of  $H_2O$  and few small pieces of carborundum to insure even boiling. Distil 50 ml or slightly more into glass-stoppered flask, using delivery tube immersed in 100 ml of boiled  $H_2O$  ( $CO_2$ -free). Using pipet, add 25 ml of the bisulfite soln, and allow to stand ca 30 min., shaking occasionally. Add excess (ca 30 ml) of the I soln, and titrate this excess with the  $Na_2S_2O_3$  soln. Run blank containing same quantities of I soln and bisulfite soln as were used with sample. Difference between titrations in ml of thiosulfate soln  $\times 0.0011$  = g of acetaldehyde in sample.

## FURFURAL—OFFICIAL

## 16.18

## REAGENT

*Standard furfural soln.*—Dissolve 1 g of redistilled furfural in 100 ml of alcohol. Prepare standards by diluting 1 ml of this soln to 100 ml with alcohol, 50% by volume. 1 ml of this soln contains 0.1 mg of furfural. (The strong furfural soln will retain its strength, but the dilute will not.)

## 16.19

## DETERMINATION

Dilute 10–20 ml of prepared distillate, 16.13, to 50 ml with furfural-free alcohol, 50% by volume. Add 2 ml of colorless aniline and 0.5 ml of HCl (sp. gr. 1.125) and keep 15 min. in water bath at ca 15°. Prepare standards of known strength and blanks in same way. Comparison standards found most convenient for use contain 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 mg of furfural.

DETECTION OF ACETONE, KETONES, ISOPROPANOL AND  
TERTIARY BUTYL ALCOHOL—TENTATIVE

## 16.20

## REAGENT

*Mercuric sulfate soln.*—Mix 5 g of yellow  $HgO$  with 40 ml of  $H_2O$ , add with stirring 20 ml of  $H_2SO_4$  and 40 ml of  $H_2O$ , and stir until completely dissolved.

## 16.21

## DETERMINATION

To 2 ml of distillate, 16.13, add 3 ml of  $H_2O$  and 10 ml of the  $HgSO_4$  soln. Heat on boiling water bath 3 min. White or yellow precipitate forming within 3 min. indicates presence of one or more of above-mentioned compounds. Disregard any pre-



precipitate forming after 3 min. on boiling water bath. If no precipitate forms, isopropanol may still be present. Then proceed as follows: Place 8 g of  $\text{CrO}_3$  in 100 ml Kohlrausch flask, and add 15 ml of  $\text{H}_2\text{O}$  and 2 ml of  $\text{H}_2\text{SO}_4$ . Connect flask with reflux condenser and add very slowly thru condenser 5 ml of sample. Boil under reflux condenser 30 min., then cool and distil off 2 ml, collecting distillate in 10 ml graduated cylinder. Mix, and test as directed previously.

#### FUSEL OIL—TENTATIVE

##### 16.22

##### REAGENTS

(a) *Purified carbon tetrachloride*.—Mix in separator crude  $\text{CCl}_4$  with 1/10 its volume of  $\text{H}_2\text{SO}_4$ , shake thoroly at frequent intervals, and allow to stand overnight. Wash free of acid and impurities with tap  $\text{H}_2\text{O}$ , remove  $\text{H}_2\text{O}$ , add an excess of  $\text{NaOH}$  soln, and distil the  $\text{CCl}_4$ .

(The refuse  $\text{CCl}_4$  after titration is purified for further work by collecting in large bottle, adding  $\text{NaOH}$  soln (1+1), shaking, washing with tap water until washings are neutral to phenolphthalein, and distilling.)

(b) *Oxidizing soln*.—Dissolve 100 g of  $\text{K}_2\text{Cr}_2\text{O}_7$  in 900 ml of  $\text{H}_2\text{O}$  and add 100 ml of  $\text{H}_2\text{SO}_4$ .

##### 16.23

##### DETERMINATION

To 50 ml of sample in 500 ml Erlenmeyer flask add 50 ml of  $\text{H}_2\text{O}$  and 20 ml of 0.5 *N*  $\text{NaOH}$ , and connect flask with condenser, using standard taper ground-glass connections, and distil off 90 ml, slowly at first, then more vigorously toward end; add 25 ml of  $\text{H}_2\text{O}$  and continue distillation until an additional 25 ml is collected.

Whenever aldehydes are present in excess of 15 parts/100,000, add to distillate 0.5 g of metaphenylenediamine hydrochloride, boil under reflux condenser an hour, distil 100 ml, add 25 ml of  $\text{H}_2\text{O}$ , and continue distillation until an additional 25 ml is collected. Approximately saturate distillate with finely ground  $\text{NaCl}$  and add saturated  $\text{NaCl}$  soln until sp. gr. is 1.10. Extract this  $\text{NaCl}$  soln 4 times with the purified  $\text{CCl}_4$ , using 40, 30, 20, and 10 ml, respectively, and wash combined  $\text{CCl}_4$  extracts 3 times with 50 ml portions of saturated  $\text{NaCl}$  soln, and twice with saturated  $\text{Na}_2\text{SO}_4$  soln. Transfer  $\text{CCl}_4$  layer to flask containing 50 ml of the oxidizing soln, and boil 8 hours under reflux condenser.

Add 100 ml of  $\text{H}_2\text{O}$  and distil until only ca 50 ml remains. Add 50 ml of  $\text{H}_2\text{O}$  and again distil until 35–50 ml is left. Use extreme care to prevent oxidizing mixture from burning and baking on side of distilling flask. Distillate should be water white; if it is colored discard it and repeat determination. Titrate distillate with 0.1 *N*  $\text{NaOH}$ , using phenolphthalein indicator. 1 ml of 0.1 *N*  $\text{NaOH}$  = 0.0088 g of amyl alcohol.

Conduct blank determination upon 100 ml of  $\text{CCl}_4$ , beginning blank at that point of procedure immediately after extraction and just before washings with  $\text{NaCl}$  and  $\text{Na}_2\text{SO}_4$  solns.

##### 16.24

##### SUGARS—OFFICIAL.—See Chap. 34

##### METHANOL

##### *Modified Denigès Method (3)—Tentative*

##### 16.25

##### APPARATUS (4)

Use a still having column 475–500 mm long and 15 mm diam. packed with small, single-turn glass helices and enclosed in glass tube of ca 30 mm diam. (See, Fig. 23 p. 199).

## 16.26

## REAGENTS

(a) *Potassium permanganate soln.*—Dissolve 3 g of  $\text{KMnO}_4$  and 15 ml of  $\text{H}_3\text{PO}_4$  in 100 ml of  $\text{H}_2\text{O}$ . Renew soln every 4 weeks.

(b) *Oxalic-sulfuric acid soln.*—Dissolve 5 g of  $\text{H}_2\text{C}_2\text{O}_4$  in 100 ml of  $\text{H}_2\text{SO}_4$  (1+1).

(c) *Modified Schiff reagent.*—Dissolve 0.2 g of Kahlbaum rosaniline HCl in ca 120 ml of hot  $\text{H}_2\text{O}$ . Cool, and add 2 g of  $\text{Na}_2\text{SO}_3$  previously dissolved in 20 ml of  $\text{H}_2\text{O}$ . Add 2.0 ml of HCl, dilute soln to 200 ml, and place in refrigerator at least 24 hours before using. Renew reagent every 4 weeks.

## 16.27

## PREPARATION OF SAMPLE

Make preliminary test as directed under 16.28, using 0.5% standard to ascertain whether more than that quantity of methanol is present (necessary only on unknown samples suspected of being recovered alcohol denatured with methanol). If more than 0.5% methanol is present, dilute to that concentration, or less, with ca 50% ethyl alcohol. Distil 25 ml portion, using fractionating column, 16.25; keep column under total reflux for ca 30 min., then draw off distillate continuously at rate of ca 20 drops/min. until ca 8.5 ml has been collected. U tube should always be full, and ratio of reflux to takeoff should be at least 5 to 1. (This should produce distillate containing ca 94% total alcohols.) Dilute distillate to 22% total alcohols and then make to any convenient volume with 22% ethyl alcohol.

## 16.28

## DETERMINATION

Transfer 0.25 ml of diluted alcoholic soln, 16.27, to 6" Nessler tube containing 4.75 ml of  $\text{H}_2\text{O}$ . Add 2 ml of the  $\text{KMnO}_4$  soln, allow to stand 10 min. with occasional shaking without inverting the tube, and then add 2 ml of the oxalic-sulfuric acid soln. Add 5 ml of the modified Schiff reagent, mix by inverting tube 3 times, stopper, and allow to stand 1 hour. Compare depth of color with known standards analyzed at same time. Convenient range for standards is 0.02–0.12% methanol by volume (in increments of 0.01% for best results). Still more accurate results may be obtained by use of neutral wedge photometer in connection with curve obtained at definite temp. by using the standards mentioned and a light filter transmitting light at 560 or 580  $\text{m}\mu$ . Spectrophotometer may also be used provided same temp. conditions are observed and the curves made at 580 or 600  $\text{m}\mu$ , plotting negative log of transmittancy as ordinates and the standards as abscissas. Use 22% ethyl alcohol in comparison standards.

## 16.29

*Immersion Refractometer Method (5)—Official*

Determine Zeiss immersion refractometer reading at  $17.5^\circ$  of distillate obtained in determination of alcohol. If, on reference to the table, 16.30, refractometer reading shows a sp. gr. agreeing with that obtained in alcohol determination, it may be assumed that no methyl alcohol is present. If, however, there is present an appreciable quantity of methyl alcohol, low reading will at once indicate that fact. If absence from the soln of refractive substances other than  $\text{H}_2\text{O}$  and the alcohols is assured, this difference in refraction is conclusive of presence of methyl alcohol.

Addition of methyl alcohol to ethyl alcohol decreases refractive index in direct proportion to quantity added; hence the quantitative calculation is made by interpolation in the table, 16.30, of the figures for pure ethyl and methyl alcohols of the same sp. gr. as the sample.

*Example.*—Distillate has sp. gr. at  $15.56^\circ$  of 0.9625 and refractometer reading at  $17.5^\circ$  of 43.1. By interpolation in the table, the readings for ethyl and methyl alcohol

at this gravity are 65.2 and 31.7, respectively, and the difference is 33.5;  $65.2 - 43.1 = 22.1$ ;  $(22.1 + 33.5)100 = 66.0$ , showing that 66.0% of total alcohol present is methyl alcohol.

**16.30** *Scale readings on Zeiss immersion refractometer at 17.5°, corresponding to specific gravities of ethyl and methyl alcohol solutions*

SP. GR. 15.56° 15.56°	SCALE READINGS		DIFFER- ENCES	SP. GR. 15.56° 15.56°	SCALE READINGS		DIFFER- ENCES
	ETHYL ALCOHOL	METHYL ALCOHOL			ETHYL ALCOHOL	METHYL ALCOHOL	
1.0000	15.0	15.0	0.0	.9720	51.5	27.0	24.5
.9990	15.8	15.3	0.5	.9710	53.0	27.5	25.5
.9980	16.6	15.6	1.0	.9700	54.6	28.1	26.5
.9970	17.5	15.9	1.6	.9690	56.1	28.7	27.4
.9960	18.5	16.2	2.3	.9680	57.6	29.2	28.4
.9950	19.4	16.5	2.9	.9670	59.1	29.6	29.5
.9940	20.4	16.9	3.5	.9660	60.6	30.1	30.5
.9930	21.4	17.2	4.2	.9650	62.0	30.6	31.4
.9920	22.5	17.5	5.0	.9640	63.3	31.0	32.3
.9910	23.6	17.9	5.7	.9630	64.6	31.5	33.1
.9900	24.7	18.2	6.5	.9620	65.8	31.9	33.9
.9890	25.9	18.6	7.3	.9610	67.0	32.4	34.6
.9880	27.1	19.0	8.1	.9600	68.1	32.8	35.3
.9870	28.4	19.5	8.9	.9590	69.2	33.3	35.9
.9860	29.6	19.9	9.7	.9580	70.2	33.7	36.5
.9850	31.0	20.4	10.6	.9570	71.2	34.1	37.1
.9840	32.4	20.8	11.6	.9560	72.1	34.5	37.6
.9830	33.8	21.3	12.5	.9550	73.0	34.9	38.1
.9820	35.2	21.8	13.4	.9540	73.8	35.3	38.5
.9810	36.7	22.3	14.4	.9530	74.6	35.6	39.0
.9800	38.3	22.8	15.5	.9520	75.4	35.9	39.5
.9790	39.9	23.4	16.5	.9510	76.2	36.2	40.0
.9780	41.5	24.0	17.5	.9500	76.9	36.5	40.4
.9770	43.1	24.5	18.6	.9490	77.6	36.8	40.8
.9760	44.8	25.0	19.8	.9480	78.3	37.0	41.3
.9750	46.5	25.5	21.0	.9470	79.0	37.3	41.7
.9740	48.2	26.0	22.2	.9460	79.7	37.6	42.1
.9730	49.8	26.5	23.3				

The scale readings are applicable only to instruments calibrated in the arbitrary scale units proposed by Pulfrich, *Z. angew. Chem.*, 1899, p. 1168. According to this scale,  $14.5 = 1.33300$ ,  $50.0 = 1.34650$ , and  $100.0 = 1.36464$ . If the instrument used is calibrated in other arbitrary units, the refractive index corresponding to the observed reading can be converted into the equivalent Zeiss reading by referring to 44.24.

*Tetramethylammonium Iodide Method (6)—Tentative*

**16.31**

PREPARATION OF SAMPLE

Transfer to distilling flask a quantity of sample that contains 20–25 ml of absolute alcohol and distil slowly, collecting distillate in 50 ml volumetric flask. When nearly to mark, disconnect receiver and adjust to mark at room temp. with  $H_2O$ .

**16.32**

APPARATUS (FIG. 24)

Connect by stopcock (C), reaction flask (A) with bulb holding ca 50 ml and side inlet tube (B) to reservoir (D) of ca 25 ml capacity. Reaction flask also has second side tube (E) thru which  $CO_2$  is conducted to help carry over iodides into receiving flask (F) and thru condenser (G), which is surrounded by 12" water jacket (H). Maintain  $H_2O$  in jacket at 50–55° by means of  $H_2O$  in flask (I) heated by burner (J). Surround reaction flask by bath (K), which contains ice and  $H_2O$  at beginning of operation and is later heated by a flame and maintained at 75–80° during remainder of

determination. By means of ground-glass joint connect outlet tube of condenser to tube M, which extends to bottom of receiving flask thru one hole of 2-holed rubber stopper. Have tube N pass thru second hole of same stopper and thence to empty 50 ml Erlenmeyer flask (O), from which a second tube (P) passes below surface of dilute  $\text{H}_2\text{SO}_4$  contained in second 50 ml Erlenmeyer flask (Q), which is also fitted with outlet tube (R) leading to surrounding atmosphere. Convey overflow from condenser to beaker (S) thru tube (T), passing thru stopper (U), which also holds

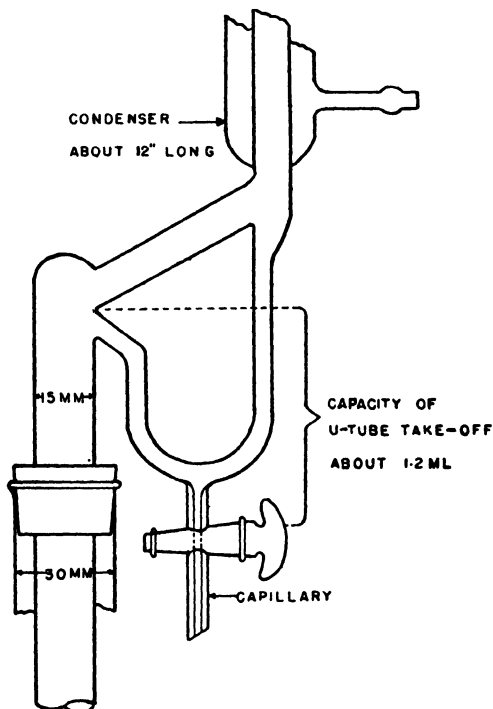


FIG. 23.—UPPER PART OF STILL AND TAKE-OFF

thermometer (V). Keep receiving flask (125 ml Erlenmeyer) cold by immersing in bath (W) containing ice and  $\text{H}_2\text{O}$ .

To collect precipitate, use sintered-glass filtering crucibles similar to Jena No. 1G4.

### 16.33

#### REAGENTS

(a) *Trimethylamine soln.*—Cool 100 g of anhydrous trimethylamine in sealed container below boiling point ( $\pm 3^\circ$ ) with ice and NaCl or by placing overnight in cold room maintained at temp. below freezing. Similarly, cool ca 1 liter of absolute alcohol and a 1 liter graduated flask. Transfer ca 700 ml of the absolute alcohol to flask, open container of trimethylamine, and transfer liquid to flask, washing out vessel with small portions of the cold absolute alcohol. Fill flask to within 50–75 ml of mark with the alcohol, mix, warm gradually to temp. of laboratory, fill to mark with absolute alcohol, and mix thoroly.

(b) *Wash soln.*—Place ca 0.25 g of tetramethylammonium iodide, obtained from a determination of methoxyl, in 500 ml flask, fill to convenient height with absolute alcohol, stopper, and shake to saturate liquid with the salt. Filter soln as needed thru white ribbon filter paper.

(c) *Carbon dioxide.*—Obtain from tank fitted with reducing valve and rubber tube connected to reaction flask.

## 16.34

## DETERMINATION

Raise temp. of  $H_2O$  in condenser jacket (H) to  $50-55^\circ$  by means of flame under flask (I). Place 15 g of I and 2 g of red P in reaction flask A, attach flask to apparatus, and surround with bath of ice and  $H_2O$  (K). Introduce 2.5 ml of alcohol into reaction flask thru reservoir (D). Measure into reservoir 10–20 ml of sample, which should contain not more than 0.160 g of methyl alcohol nor more than 7 ml of ethyl alcohol. Place 25 ml of wash soln in receiving flask (F), connect flask to apparatus by means of tube M, and surround it with the bath containing ice and  $H_2O$  (W). Attach  $CO_2$  tank and so adjust as to make it possible to start current of gas at moment's notice. Stir ice and  $H_2O$  in bath to cool reaction flask to as near  $0^\circ$  as possible. Adjust stopcock (C) so that sample will flow slowly down cold sides of reaction flask (A). (Addition of 10 ml of sample should require 3–5 min.) Stir ice and  $H_2O$  constantly during addition. When all sample has run in, wash sides of reservoir with two or three small portions of  $H_2O$ , using 5–10 ml, and add washings to contents of reaction flask. Fill reservoir with  $H_2O$  to prevent leakage. Remove ice from bath, leaving the cold  $H_2O$ . (Mixture in reaction flask should now consist of two layers: bottom one, dark red; upper one, colorless or nearly so. Layers will gradually mingle. If vapors of HI are seen to rise from surface of liquid, due to violence of reaction during mixing of two layers, cool by stirring bath, adding small piece of ice if necessary to prevent mixture from boiling or giving off acid vapors too rapidly.)

When contents of reaction flask have become homogeneous except for the floating particles of P, place burner under bath and heat fairly rapidly to  $75^\circ$ . During heating add to receiving flask 10 ml of trimethylamine soln and 25 ml of wash soln, and attach flask containing the dilute  $H_2SO_4$  to prevent the trimethylamine from escaping into air. When contents of reaction flask begin to boil, turn on the  $CO_2$  at rate of ca 50 bubbles/min., counting them as they rise from tube in receiving flask. Allow distillation to proceed 1.5–2 hours, maintaining temp. of bath at  $75-80^\circ$ , jacket (H) at  $50-55^\circ$ , and bath (W) at or near  $0^\circ$ .

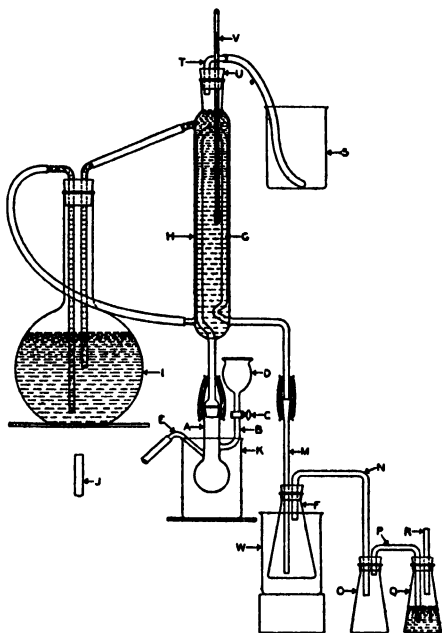


FIG. 24.—APPARATUS FOR DETERMINATION OF METHYL ALCOHOL

Disconnect receiving flask and wash out tube (M) with 10–15 ml of wash soln, using rubber policeman to scrub off any crystals that may adhere to outside of tube and small glass rod to remove crystals from inside, if necessary. Stopper flask and let stand at room temp. overnight. (Stopper should remain loose until contents of flask reach temp. of room.) Filter mixture on weighed sintered glass crucible, using 35–40 ml of wash soln to transfer precipitate from flask to crucible. (If filtrate becomes cloudy or crystals separate out, do not be concerned since soln may contain large quantities of trimethylethylammonium iodide, which is only soluble to extent of ca 4 g/100 ml of absolute alcohol, and large quantities of crystals are deposited due to rapid evaporation of alcohol in suction flask.) At this point wash off outside of crucible with 95% alcohol to remove the crystals of trimethylethylammonium iodide that have formed on bottom and lower sides of crucible, and suck dry. Now wash contents and inside of crucible three times in following manner: Turn off suction, add ca 5 ml of wash soln by pouring down sides of crucible, and mix liquid with crystals by rotating crucible or by stirring contents with small glass rod or fine stream of wash soln from wash bottle. Wash off rod. Cover crucible with small watch-glass and let stand 2–3 min. Suck dry. After third washing, remove crucible from holder, and carefully wash off outside of crucible with 95% alcohol, sucking dry at once if any liquid gets on bottom of sinter. Dry at 100–110° 1 hour, cool in desiccator, and weigh. Weight of precipitate  $\times 0.15933$  = weight of methyl alcohol in portion taken for analysis.

In determining 5 mg or less, Pregl filtering tubes may be used to advantage.

**16.35**                      **COLORING MATTERS—TENTATIVE.—***See* Chap. 21

**16.36**                      **WATER-INSOLUBLE COLOR—TENTATIVE**

Evaporate 50 ml of sample just to dryness on steam bath. Take up with ca 15 ml of cold  $H_2O$ , filter, and wash until filtrate amounts to nearly 25 ml. To this filtrate add 25 ml of absolute alcohol, or 26.3 ml of alcohol, and make up to 50 ml with  $H_2O$ . Mix thoroly and compare in colorimeter with original material. Calculate from these readings percentage of color insoluble in  $H_2O$  (100%—color observed).

**16.37**                      **COLOR INSOLUBLE IN AMYL ALCOHOL (?)—TENTATIVE**

Evaporate 50 ml of sample just to dryness on steam bath. Dissolve residue in  $H_2O$  and alcohol and make to volume of 50 ml, using total volume of 26.3 ml of alcohol. Place 25 ml of this soln in separator and add 20 ml of freshly shaken Marsh reagent (100 ml of pure amyl alcohol, 3 ml of sirupy  $H_3PO_4$ , and 3 ml of  $H_2O$ ), shaking lightly so as not to form an emulsion. Allow layers to separate and repeat this shaking and standing twice. After layers have separated completely draw off lower or aqueous layer, which contains the caramel, into 25 ml cylinder and make up to volume with alcohol, 50% by volume. Compare this soln in colorimeter with the untreated 25 ml. Calculate from this reading percentage of color insoluble in amyl alcohol.

**ARTIFICIAL COLORS**

**16.38**                      ***Marsh Test—Tentative***

To 10 ml of sample in 20 ml test tube, add sufficient Marsh reagent, 16.37, to nearly fill tube, and shake several times. Allow layers to separate. Color in lower layer indicates that sample has been colored with caramel, a coal tar dye, or with extractive material from uncharred white oak chips.

In absence of any color, test 10 ml in same manner, using sufficient fusel oil, amyl

alcohol, or pentasol to nearly fill tube, and shaking several times. A deeply colored lower layer indicates a coal tar dye. Ascertain its identity as directed under Chap.

21. To confirm caramel apply one or more of following tests:

## 16.39

*Modified Marsh Test (8)—Official*

Place 25 ml of spirits in 150 ml beaker marked to show volumes of 13 ml and 25 ml; add 0.5 ml of acetic acid and 0.75 g of Zn acetate crystals, and mix. When crystals are nearly dissolved, boil down rapidly over flame to 13 ml mark, stirring frequently to prevent bumping or spattering. If liquid should inadvertently go below 13 ml mark, fill to that mark with  $H_2O$ , and set aside to cool. When cooled to room temp. fill to 25 ml mark with alcohol, mix, and allow to stand 2–3 min. Mix again, and filter thru double filter (folded or S. & S. white ribbon). Mix filtrate and transfer 6 ml to 6" test tube; add 12 ml of Marsh reagent, 16.37; and mix thoroly until voluminous white precipitate that forms when liquids first mix goes back into soln. Allow to stand until layers separate, then pour off 4 ml of upper layer into graduated cylinder and in its place in test tube pour 4 ml of 88% grade ethyl acetate; mix, and allow to stand until layers separate. Dark brown color in lower layer indicates that caramel is present. If, however, lower layer is colorless and a positive Marsh test was obtained under 16.38, coloring from uncharred white oak chips is indicated. If lower layer has reddish shade, coal tar colors may be present. Confirm presence of coal tar color by transferring some of remaining filtrate to porcelain dish and adding a few drops of HCl. If coal tar colors are present, soln may become red. For further confirmation add  $SnCl_2$  soln, which will decolorize the soln. Use clear light of open window as background for examining colors obtained in these tests.

## 16.40

*Cyclohexanol Test—Tentative*

To 10 ml of sample diluted to below 80° proof in 20 ml test tube add sufficient cyclohexanol reagent (mixture of 50 volumes each of cyclohexanol and methyl propyl ketone and 3 volumes each of  $H_3PO_4$  and  $H_2O$ ) to nearly fill tube, and invert several times. Allow layers to separate. Color in lower layer indicates that sample has been colored with caramel or a coal-tar dye.

## 16.41

*Milos Test—Official.—See 15.38—15.39*

## TANNIN—TENTATIVE

## 16.42

## REAGENTS

(a) *Folin-Denis reagent.*—To 750 ml of  $H_2O$  add 100 g of  $Na_2WO_4 \cdot 2H_2O$ , 20 g of phosphomolybdic acid, and 50 ml of  $H_3PO_4$ . Reflux 2 hours, cool, and dilute to 1 liter.

(b) *Saturated sodium carbonate soln.*—To each 100 ml of  $H_2O$  add 35 g of anhydrous  $Na_2CO_3$ , dissolve at 70–80°, and allow to cool overnight. Seed the supersaturated soln with a crystal of  $Na_2CO_3 \cdot 10H_2O$ , and after crystallization filter thru glass wool.

(c) *Standard tannic acid soln.*—Dissolve exactly 100 mg of tannic acid in 1 liter of  $H_2O$ . Prepare a fresh soln for each determination.

## 16.43

## DETERMINATION

Place 0.25–1.00 ml of whisky in Nessler tube containing ca 90 ml of  $H_2O$ . Add 1.0 ml of the Folin-Denis reagent and make up to mark with  $H_2O$ . Then add 5.0 ml of the  $Na_2CO_3$  soln and shake well. After 10–15 min. compare the blue color developed with standards made in the same way at the same time containing 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6, 1.8, 2.0, and 2.4 ml of the standard tannic acid soln.

## CORDIALS AND LIQUEURS—TENTATIVE\*

## 16.44

## PHYSICAL EXAMINATION

Note and record following: (1) Appearance, whether bright or turbid and whether there is any sediment; (2) color and depth of color; (3) odor; (4) taste.

## 16.45

## SPECIFIC GRAVITY.—See 14.3

## 16.46

## ALCOHOL

(a) *By weight*.—Proceed as directed under 16.7.

(b) *By volume*.—Proceed as directed under 16.6.

## METHANOL

## 16.47

## PREPARATION OF SAMPLE

Measure into distilling flash such a quantity of sample as contains 20–25 ml of absolute alcohol, add sufficient  $H_2O$  to make total volume ca 100 ml, and distil, collecting ca 50 ml of distillate. To distillate add 4 g of NaCl for each 10 ml of  $H_2O$ , and allow to stand several hours to reach saturation point. Transfer to separator, using ca 10 ml of saturated NaCl soln to wash out container, and shake with 25 ml of petroleum benzine. When separation is complete, transfer the  $H_2O$  soln to second separator containing 25 ml of petroleum benzine; shake, and transfer the  $H_2O$  soln to third separator, also containing 25 ml of petroleum benzine; shake and when separation is complete, drain off  $H_2O$  soln into 200 ml distilling flask. In meantime, add to first separator 25 ml of saturated NaCl soln and follow sample thru with this soln, finally adding washings to sample soln in distilling flask. Repeat this operation with a second 25 ml portion of saturated NaCl soln, finally adding this also to distilling flask. Distil mixture into 50 ml volumetric flask, using suitable adapter. When 48–49 ml has distilled over, disconnect apparatus and fill flask to mark with  $H_2O$ . Mix, and determine methyl alcohol as directed under 16.34.

## 16.48

## ALDEHYDES

Measure 100–200 ml of sample into distillation flask. If solid content is 25 g/100 ml or less, add 12.5–25 ml of  $H_2O$ ; if greater than 25 g/100 ml, add 5 ml of  $H_2O$  for each 10 g of solid matter present; distil slowly, collecting volume of distillate equal to that of sample, and proceed as directed under 16.15 or 16.17.

## 16.49

## FURFURAL

Treat portion of prepared distillate, 16.48, and proceed as directed under 16.19.

## 16.50

## FUSEL OIL

Treat 50 ml of prepared distillate, 16.48, and proceed as directed under 16.23.

## 16.51

## TOTAL SOLIDS

(a) *From sp. gr. of dealcoholized sample—tentative*.—See 14.6.

(b) *By evaporation—tentative*.—See 34.5.

(c) *From refractive index of dealcoholized sample—official*.—Restore residue from alcohol determination to original volume by making necessary evaporation or dilution. Determine refractometer reading of the soln at 20° and obtain corresponding percentage of dry substance. From 44.3, ascertain sp. gr. corresponding to percentage of dry substance found and multiply by percentage dry substance to obtain grams

\* Unless otherwise indicated.



of total solids/100 ml of sample. To obtain percentage of total solids in sample, divide grams of total solids/100 ml by sp. gr. of sample, 14.3.

## 16.52

## GLYCEROL

(a) *Products containing 5 g/100 ml or less of total solids.*—See 15.5 or 15.6.

(b) *Products containing more than 5 g/100 ml of total solids.*—Measure into porcelain dish such a quantity of sample (not to exceed 100 ml) as contains 25 g or less of solid matter and evaporate on steam bath to remove alcohol. Transfer to 500 ml Erlenmeyer flask, using such a quantity of  $H_2O$  that final volume will be ca 100 ml, and proceed as directed in 15.7.

## SUCROSE

## 16.53

*Method I. By Polarization*

Pipet into evaporating dish volume of sample equivalent to 52 g, as calculated from sp. gr., 14.3; exactly neutralize with normal NaOH, calculating quantity required from determination of acidity, 16.62; evaporate on steam bath to remove alcohol; transfer to 200 ml flask; and proceed as directed under 34.23 or 34.24, beginning "add necessary clarifying reagent, etc."

## 16.54

*Method II. By Reducing Sugars before and after Inversion*

Approximate sugar content of sample from total solids, 16.51, and pipet into porcelain dish such a quantity of sample as will contain 5–7 g of sugars; exactly neutralize with standard NaOH soln, calculating quantity required from acidity, and evaporate on steam bath to remove alcohol. Transfer to 200 ml volumetric flask, clarify with neutral Pb acetate soln, 34.19 (d), remove excess Pb with K oxalate, and proceed as directed under 34.30, using method given under 34.39, for determination of reducing sugars.

## 16.55

## ASH

Proceed as directed under 34.9 or 34.10, using 25 ml of sample.

## 16.56

## SOLUBLE AND INSOLUBLE ASH

Using ash obtained under 16.55, proceed as directed under 34.13.

## 16.57

## ALKALINITY OF SOLUBLE ASH

Using soluble ash obtained under 16.56, proceed as directed under 34.14.

## 16.58

## ALKALINITY OF INSOLUBLE ASH

Using insoluble ash obtained under 16.56, proceed as directed under 34.15.

## 16.59

## PHOSPHORIC ACID

Evaporate 25 ml of sample to sirupy consistency on steam bath; add 7.5 ml of  $Mg(NO_3)_2$  soln, 2.7 (e); mix thoroly, continue evaporation as far as possible on steam bath, and proceed as directed under 12.35, beginning "heat on electric hot plate ( $180^\circ$ )."

Determine  $P_2O_5$  on soln obtained as directed under 2.9 or 2.12.

## 16.60

## CARAMEL.—See 16.38–16.41

## 16.61

## COAL TAR COLORS.—See Chap. 21

## 16.62

## TOTAL ACIDITY

Place ca 600 ml of  $H_2O$  in 800 ml beaker, add ca 1 ml of phenolphthalein indicator, and titrate to pink color with 0.1  $N$   $NaOH$ . Add 10–20 ml of sample (unless this quantity gives soln such a deep color that it will obscure end point, in which case 5 ml may be used) and titrate to pink color comparable to that of soln before sample was added. Calculate acidity as g/100 ml of sample in terms of predominating acid present in sample.

## 16.63

## PRELIMINARY PROCEDURE FOR CHARACTERISTIC ACIDS

Measure out such a volume of sample as contains not more than 30 g of solid matter and not more than 200 mg of the acid to be determined, as calculated from acidity; evaporate to ca 30 ml, add 6 ml of 1  $N$   $NaOH$ , and let stand at least 3 hours. Add 8 ml of 1  $N$   $H_2SO_4$ , transfer to 250 ml volumetric flask, using 10 ml of  $H_2O$  and sufficient alcohol to fill flask to mark; mix and let stand 15 min. Filter thru thin layer of absorbent cotton, protecting liquid against evaporation. Transfer 200 ml of filtrate to centrifuge bottle and proceed with determination of the acid as directed.

## 16.64

## TARTARIC ACID

Using material in centrifuge bottle, proceed as directed under 26.33 or 26.35.

## 16.65

## CITRIC ACID

Using material in centrifuge bottle, proceed as directed under 26.37.

## 16.66

## ACTIVE MALIC ACID

Using material in centrifuge bottle, proceed as directed under 26.40.

## 16.67

## INACTIVE MALIC ACID

Using material in centrifuge bottle, proceed as directed under 26.43.

## 16.68

## VOLATILE ESTERS—OFFICIAL

Measure 100–500 ml of sample into distilling flask and steam distil as directed under 13.26, collecting volume of distillate at least twice as great as volume of alcohol contained in sample. (If determination 16.69 is to be made, use 500 ml sample.) Disconnect apparatus and wash out condenser with a little  $H_2O$ . Add ca 1 ml of phenolphthalein indicator and titrate to pink color that persists at least 1 min., using 0.1  $N$   $NaOH$  or  $KOH$ . Add to soln measured excess of 25–50 ml of 0.1  $N$  alkali, reflux 1 hour, cool, and titrate excess of alkali with 0.1  $N$   $H_2SO_4$ . Calculate number of ml of 0.1  $N$  alkali used in saponification of esters as ethyl acetate. 1 ml of 0.1  $N$  alkali = 8.8 mg of  $CH_3COOC_2H_5$ .

## 16.69

## GAMMA UNDECALACTONE (QUALITATIVE) (9)—OFFICIAL

(Peach and Apricot Cordials)

Make distinctly alkaline soln obtained under 16.68 and evaporate to dryness on steam bath. Take up residue in ca 25 ml of  $H_2O$ , transfer to separator, acidify with  $H_2SO_4$  (1+1), let stand 10 min. to permit lactones to form, and extract 3 times with ca 20 ml of ether. Unite ether extracts and wash 3 times by shaking with 10 ml portions of normal  $Na_2CO_3$  soln. Permit ether soln to evaporate spontaneously in small beaker. To residue add few drops of  $N_2H_4 \cdot H_2O$  soln (42% in  $H_2O$ ) and mix

thoroly; if white solid matter separates out in a few minutes, gamma undecalactone is present. Allow mixture to stand 15–20 min., place on steam bath, and heat until ammoniacal odor is no longer evident. Add 1 ml of normal butyl alcohol and warm until clear soln is obtained, adding a few additional drops of the alcohol if necessary to dissolve residue completely. Remove from steam bath and permit butyl alcohol to evaporate spontaneously. (This usually occurs overnight, but longer time may be necessary if much butyl alcohol has been used.) Examine colorless or slightly yellowish crystals under microscope. If optical properties of crystals, 16.70, correspond to those of hydrazino- $\gamma$ -undecalactone, presence of gamma undecalactone is indicated. (Hydrazino- $\gamma$ -undecalactone has a characteristic odor similar to that of the lactone itself.)

#### 16.70 OPTICAL PROPERTIES OF HYDRAZINO- $\gamma$ -UNDECALACTONE

In ordinary light the substance is seen to consist of lath-like rods, many of them more or less split at ends. In parallel polarized light (crossed nicols), substance is characterized by not extinguishing sharply, most of rods remaining essentially bright when stage is rotated. Occasionally there are found crystals that extinguish sharply, have square ends, and show straight extinction and negative elongation. In convergent polarized light (crossed nicols) partial biaxial interference figures, usually showing one optic axis up or slightly inclined to normal, are of frequent occurrence. The refractive indices, as determined by immersion method, are as follows:  $\alpha = 1.483$  (not common);  $\beta = 1.525$  (most frequently occurring of indices and shown lengthwise on rods);  $\gamma = 1.555$  (occurring crosswise on rods which show straight extinction and negative elongation); all  $\pm 0.003$ .

#### BENZALDEHYDE (10)—OFFICIAL, FIRST ACTION

##### 16.71 REAGENT

*Phenylhydrazine soln.*—Add 1.5 ml of acetic acid and 1 ml of newly distilled phenylhydrazine to 20 ml of  $H_2O$  and filter thru moistened, double, white ribbon filter.

##### 16.72 DETERMINATION

Measure into distilling flask such a quantity of sample as contains 30 ml of absolute alcohol, dilute to such a volume that mixture will contain 300 ml of  $H_2O$  in addition to that required to dissolve sugar present (1 g of sugar requires 0.5 ml of  $H_2O$ ), and distil 300 ml into 500 ml Erlenmeyer flask. Add 10 ml of phenylhydrazine soln and shake 5 min. Filter on Gooch with thin mat, and wash with  $H_2O$  and finally with two 10 ml portions of 10% alcohol. Dry in vacuum desiccator over  $H_2SO_4$  24 hours, excluding light, or at  $70^\circ$  under 100 mm or less of pressure 2 hours. Wt. of precipitate  $\times 0.5408$  = wt. of benzaldehyde.

##### 16.73 THUJONE (11)—TENTATIVE

To 500 ml of absinthe add 1 ml of freshly distilled aniline and 1 ml of sirupy phosphoric acid, and reflux 30 min. on steam bath. Distil off two 100 ml portions, reject first, and test second for thujone as follows:

Add 0.5 g of semicarbazide hydrochloride and 0.6 g of anhydrous Na acetate (or 1.0 g of the crystallized salt) and allow mixture to stand overnight. Distil off alcohol at as low a pressure as possible. Steam distil to remove essential oils and other volatile material, collect, and reject ca 15 ml of distillate. Wash down condenser with a little alcohol and with  $H_2O$ . Cool sample, add 1 ml of  $H_2SO_4$  (1+1) and again steam distil, this time collecting 20 ml of distillate in cylinder. Pour distillate into small

separator, and add 20 ml of ether, using receiver as the measure. Shake, and separate the ether soln. Add 10 ml of 65% alcohol and allow ether to evaporate spontaneously. When all ether has evaporated, note odor of residue. Odor of thujone will be apparent if 2 mg or more is present in soln, provided it is not masked by presence of other odoriferous substances. Make modified Legal test as follows:

To soln obtained as directed above, add 1 ml of 10%  $\text{ZnSO}_4$  soln and 0.25 ml of freshly prepared Na nitroprusside soln (0.1 g/ml of  $\text{H}_2\text{O}$ ). Slowly, with constant stirring, add 2 ml of 5% NaOH soln. Allow to stand 1-2 min. Add 1.5 ml of acetic acid and mix. Precipitate of raspberry red color (resembling alcohol precipitate of red fruit juice) shows presence of thujone. Negative test is shown by similar precipitate having appearance similar to that of alcohol precipitate from apple jelly or other light colored fruit.

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## 17. BAKING POWDERS AND BAKING CHEMICALS

### 17.1

#### PREPARATION OF SAMPLE—OFFICIAL

Remove entire sample from package, pass thru 20-mesh sieve, and mix thoroly.

#### TOTAL CARBON DIOXIDE

##### *Gravimetric Method with Knorr Apparatus (1)—Official*

### 17.2

#### APPARATUS

Connect flask by means of ground-glass joint with glass connection thru upper part of which passes a dropping funnel, and join at side with Liebig condenser. Connect mouth of funnel by means of perforated stopper with soda lime tube. Connect upper end of condenser by rubber joint with train of absorption bulbs, the first containing  $\text{H}_2\text{SO}_4$  for drying the gas passing into next bulb, which contains 33% KOH soln. Connect to third bulb containing  $\text{H}_2\text{SO}_4$  for absorption of moisture escaping from KOH bulb, then to fourth bulb, also containing  $\text{H}_2\text{SO}_4$ , as precaution to prevent moisture from air being absorbed by train. Connect last bulb to aspirator. (Many analysts prefer to replace last bulb by two U-tubes filled with sifted soda lime.)

### 17.3

#### DETERMINATION

Place 0.5–2 g of sample, quantity depending upon percentage of  $\text{CO}_2$  present, in flask, which must be perfectly dry. Close flask with stopper which carries funnel tube and tube connecting with absorption apparatus. Weigh separately second and third absorption bulbs and attach them to apparatus. If two soda lime tubes are used, weigh separately and refill first when second increases materially in weight. Nearly fill funnel tube with  $\text{H}_2\text{SO}_4$  (1+5) and place soda lime tube in position. Aspirate air thru absorption bulbs at rate of ca 2 bubbles per second. Open stopper of funnel and allow acid to run slowly into flask, taking care that evolution of gas is so gradual as not materially to increase current thru bulbs. After all acid has been introduced, close stopcock, continue aspiration, and gradually heat contents of flask to boiling. (While flask is being heated aspirator tube may be removed, altho when using ground-glass joints many analysts prefer to aspirate during entire operation.) Continue boiling a few minutes after the  $\text{H}_2\text{O}$  has begun to condense; remove flame, open stopcock, and continue aspiration while apparatus cools. Remove second and third bulbs and weigh. Increase in weight is due to  $\text{CO}_2$ .

##### *Gasometric Method (2) with Chittick Apparatus—Official*

### 17.4

#### REAGENT

*Displacement soln.*—Dissolve 100 g of  $\text{NaCl}$  of  $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$  in 350 ml of  $\text{H}_2\text{O}$ . Add ca 1 g of  $\text{NaHCO}_3$  and 2 ml of methyl orange indicator, 6.3(f), and then sufficient  $\text{H}_2\text{SO}_4$  (1+5) to make just acid (decided pink color). Stir until all  $\text{CO}_2$  is removed. This soln is used in the gas-measuring tube and leveling bulb and seldom needs to be replaced.

### 17.5

#### APPARATUS

Connect decomposition flask (A) by means of glass T-tube (B), provided with stopcock (C), to graduated gas-measuring tube (D), which in turn is connected with leveling bulb (E). For A always use 250 ml wide-mouth extraction flask of Pyrex

or other resistant glass fitted with two-holed rubber stopper, thru one hole of which passes extended tip of 25 ml buret (*F*) and thru other glass tube of same diameter as connecting T-tube. Use buret graduated in ml at 20°, numbered at 5 ml intervals, and provided with extra long tip bent to pass thru rubber stopper. Connect the glass tube leading from decomposition flask to T-tube by means of rubber tubing to permit rotation of flask. Use gas-measuring tube graduated in ml at 20°, the zero mark being placed at point 25 ml below top marking to allow for graduating upward from 0 to 25 ml and downward from 0 to 200 ml. By means of long rubber tube connect gas-measuring tube with leveling bulb, which has a capacity of ca 300 ml.

## 17.6

## DETERMINATION (3)

Weigh 1.7 g of prepared sample, 17.1, into flask *A* and connect this flask with apparatus (Fig. 25). Open stopcock *C* and by means of leveling bulb *E* bring displacement soln to 10 ml graduation above zero mark. (This 10 ml is practically equal in volume to volume of acid to be used in decomposition.) Allow apparatus to stand 1–2 min. to insure that temp. and pressure within apparatus are same as those of the room. Close stopcock, lower leveling bulb somewhat to reduce pressure within apparatus, and slowly run into decomposition flask from buret *F* 10 ml of  $\text{H}_2\text{SO}_4$  (1+5). To prevent liberated  $\text{CO}_2$  from escaping thru acid buret into air, keep displacement soln in leveling bulb at all times during decomposition at lower level than that in the gas-measuring tube. Rotate, and then vigorously agitate decomposition flask to secure intimate mixture of contents. Allow to stand 5 min. to secure equilibrium. Equalize pressure in measuring tube by means of leveling bulb and read volume of gas in tube. Observe temp. of air surrounding apparatus and also barometric pressure at the time and multiply number of ml of gas evolved by factor given in table for this temp. and pressure, 44.30. Divide corrected reading by 10 to obtain percentage by weight of  $\text{CO}_2$  in sample.

## RESIDUAL CARBON DIOXIDE (4)

## 17.7

*Gravimetric Method—Official*

Weigh 2 g of prepared sample, 17.1, into flask suitable for subsequent determination of  $\text{CO}_2$ ; add 20 ml of cold  $\text{H}_2\text{O}$ ; and allow to stand 20 min. Place flask in metal drying cell surrounded by boiling  $\text{H}_2\text{O}$  and heat,

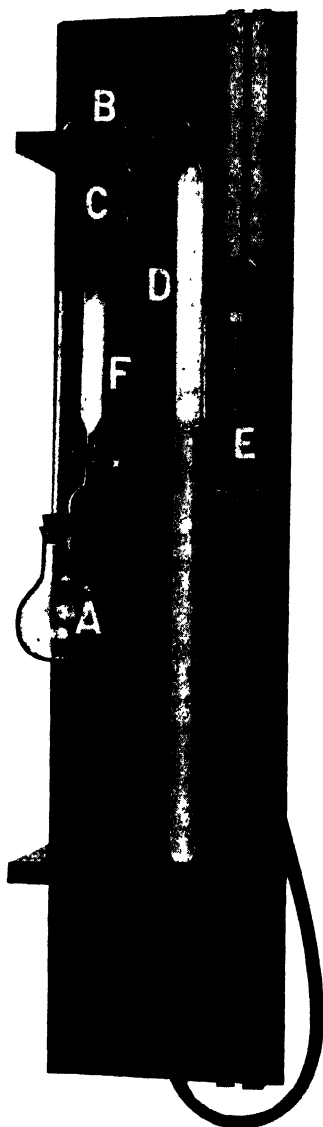


FIG. 25.—APPARATUS FOR GASOMETRIC DETERMINATION OF CARBON DIOXIDE

with occasional shaking, 20 min. To complete reaction and drive off last traces of gas from semi-solid mass, heat quickly to boiling and boil 1 min. Aspirate until air in flask is thoroly changed, and determine residual  $\text{CO}_2$  by absorption, as directed under 17.3.

**17.8***Gasometric Method (5)—Official*

Place 1.7 g of prepared sample, 17.1, in decomposition flask, A, Fig. 25; add 20 ml of  $\text{H}_2\text{O}$  and allow to stand 20 min. Place flask in metal drying cell surrounded by boiling  $\text{H}_2\text{O}$  and heat, with occasional shaking, 20 min. To complete reaction, heat quickly to boiling and boil 1 min. Cool to room temp., connect flask to apparatus described under 17.5, and determine  $\text{CO}_2$  present by treating with 10 ml of  $\text{H}_2\text{SO}_4$  (1 + 5) as directed under 17.6, using correction factors given in table, 44.30. To prevent foaming add 1–3 drops of capryl alcohol to baking powder in decomposition flask.

**17.9****AVAILABLE CARBON DIOXIDE—OFFICIAL**

Subtract residual  $\text{CO}_2$ , 17.7, from total  $\text{CO}_2$ , 17.3; or subtract 17.8 from 17.6.

**NEUTRALIZING VALUE****17.10***Of Acid-Reacting Materials Other Than Phosphates—Official*

Dissolve 1 g of sample in hot  $\text{H}_2\text{O}$  and titrate with 0.2 N NaOH, using phenolphthalein indicator. Express result as parts of  $\text{NaHCO}_3$  equivalent to 100 parts of the acid-reacting material.

**17.11***Of Monocalcium Phosphate (6)—Tentative*

Weigh 0.84 g of monocalcium acid phosphate into 375 ml casserole. Add 24 ml of cold  $\text{H}_2\text{O}$ , and after stirring for moment add exactly 90 ml of 0.1 N NaOH. Bring suspension to boil in exactly 2 min. and boil 1 min. While soln is still boiling hot add 1 drop of phenolphthalein indicator, 7.6(a), and back titrate with 0.2 N HCl until all pink color has disappeared. Boil soln 1 min. and again add 0.2 N HCl until pink color has just disappeared. Multiply number of ml of 0.2 N HCl used by 2 and subtract from 90; difference is neutralizing value, parts of  $\text{NaHCO}_3$  equivalent to 100 parts of the phosphate.

**17.12***Of Sodium Acid Pyrophosphate (7)—Tentative*

Weigh 0.84 g of sodium acid pyrophosphate and 20 g of pure NaCl into 375 ml casserole and add 25 ml of  $\text{H}_2\text{O}$  slowly while stirring. Add 90 ml of 0.1 N NaOH and 1 drop of phenolphthalein indicator, 7.6(a), and titrate with 0.2 N HCl to disappearance of pink color. If a "starch filled" or 50% neutralizing strength pyrophosphate is being titrated, use 70 ml of the NaOH. Multiply number of ml of 0.2 N HCl used by 2 and subtract from number of ml of 0.1 N NaOH used; difference is neutralizing value, parts of  $\text{NaHCO}_3$  equivalent to 100 parts of sodium acid pyrophosphate.

**17.13 TARTARIC ACID, FREE OR COMBINED (QUALITATIVE TEST) (8)—TENTATIVE**

(Applicable in presence of phosphates.)

Shake repeatedly ca 5 g of sample with ca 250 ml of cold  $\text{H}_2\text{O}$  in flask and allow insoluble portion to subside. Decant soln thru filter and evaporate filtrate to dryness. Powder residue, add few drops of 1% resorcinol soln, 34.102, and ca 3 ml of  $\text{H}_2\text{SO}_4$ , and heat slowly. Tartaric acid is indicated by a rose-red color, which is discharged on dilution with  $\text{H}_2\text{O}$ .

## CREAM OF TARTAR AND FREE TARTARIC ACID IN TARTRATE POWDERS (9)

*Total, Combined, and Free Tartaric Acid—Official*

## 17.14

## DETERMINATION

To 2.5 g of baking powder in 250 ml volumetric flask, add 100 ml of  $H_2O$  at ca  $50^\circ$ , and allow to stand at room temp. ca 30 min., shaking occasionally. Cool, dilute to mark with  $H_2O$ , shake vigorously, and filter thru large fluted paper. Pipet 2 portions of 100 ml each of the *clear* filtrate into 250 ml beakers and evaporate to ca 20 ml. To one portion add 3.5 ml of ca normal KOH. Mix well and add 2 ml of acetic acid. Again mix well and add 100 ml of alcohol, stirring constantly. Treat other portion in similar manner, but use normal NaOH instead of KOH. Then treat each mixture separately as follows: Cool to ca  $15^\circ$ , stir vigorously ca 1 min., and allow to remain in refrigerator overnight. Collect precipitate in Gooch on thin, tightly tamped pad of asbestos. Rinse beaker with ca 75 ml of ice-cold 80% alcohol, carefully washing down sides of beaker. Finally, wash sides of crucible with 25 ml of alcohol and suck dry. Transfer contents of crucible to original beaker with ca 100 ml of hot  $H_2O$  and titrate with 0.1 N alkali, using phenolphthalein indicator. Designate titer of portion treated with KOH as "A" and that treated with NaOH as "B."

## 17.15

## CALCULATIONS

Percent total tartaric acid  $= 1.5(A + 0.6)$ .

Percent cream of tartar  $= 1.88(B + 0.6)$ .

Percent free tartaric acid  $= 1.5(A - B)$ .

In above formulas "0.6" represents solubility of cream of tartar in reaction mixture in terms of 0.1 N alkali.

*Free Tartaric Acid (Direct Determination)—Official*

## 17.16

## REAGENT

*Saturated alcohol.*—To ca 50 g of purest cream of tartar (finely powdered) in Erlenmeyer flask, add ca 100 ml of alcohol and 100 ml of  $H_2O$ , shake vigorously several minutes, and allow to stand 15 min., shaking occasionally. Filter on paper in Büchner funnel, and wash with ca 200 ml of dilute alcohol (1+1), then with alcohol, and finally with ether. Dry at temp. of boiling  $H_2O$ . To 500 ml of *absolute* alcohol add ca 5 g of the purified cream of tartar and allow to stand 2 hours, shaking occasionally. If the cream of tartar has been properly purified, not more than 0.15 ml of 0.1 N alkali should be required to neutralize 100 ml of a mixture of 50 ml of  $CHCl_3$  and 150 ml of the saturated alcohol.

## 17.17

## DETERMINATION

Weigh 1.25 g of baking powder into an *absolutely dry* 200 ml volumetric flask, add 50 ml of  $CHCl_3$ , and allow to stand ca 5 min., shaking occasionally. (If, upon addition of the  $CHCl_3$ , the powder sticks to bottom of the flask, moisture is indicated and determination should be discarded.) Add 100 ml of the saturated alcohol, shake ca 5 min., and allow to stand 30 min., shaking at frequent intervals. (It is not necessary to filter the alcohol reagent.) Make to mark with the saturated alcohol, shake a few minutes, and filter thru large fluted paper. Titrate 100 ml of clear filtrate with 0.1 N alkali (phenolphthalein). The quantity (ml) of alkali used  $\times 1.2 =$  percentage of free tartaric acid.

## 17.18

*Free Tartaric Acid (Qualitative Test)—Official*

Extract 5 g of sample with absolute alcohol and evaporate the alcohol from the extract. Dissolve residue in  $NH_4OH$  (1+10), transfer to test tube, add good sized



crystal of  $\text{AgNO}_3$ , and heat gently. Tartaric acid is indicated by formation of Ag mirror. If desired, the absolute alcoholic extract may be tested as directed under 17.13.

#### STARCH

##### 17.19

##### *Direct Inversion Method—Official*

(For baking powders and baking chemicals free from calcium)

Weigh 5 g of sample into 500 ml volumetric flask and proceed as directed under 27.33.

##### 17.20

##### *Indirect Method (10)—Official*

(For baking powders and baking chemicals containing calcium)

Mix 5 g of sample with 200 ml of  $\text{HCl}$  (1+11) in 500 ml volumetric flask and allow mixture to stand an hour, shaking frequently. Filter on 11 cm hardened filter, taking care to obtain clear filtrate. Rinse flask once without attempting to remove all the starch, and wash paper twice with cold  $\text{H}_2\text{O}$ . Carefully wash starch from paper back into flask with 200 ml of  $\text{H}_2\text{O}$ . Add 20 ml of  $\text{HCl}$  (sp. gr. 1.125) and proceed as directed under 27.33. (Treatment with the  $\text{HCl}$ , without dissolving the starch, removes effectively the Ca, which otherwise would be precipitated as tartrate by the alkaline Cu soln.)

##### 17.21

##### *Modified McGill Method (11)—Tentative*

Digest 1 g of sample with 150 ml of  $\text{HCl}$  (1+11) 24 hours at room temp., with occasional shaking. Filter on Gooch crucible, wash thoroly with cold  $\text{H}_2\text{O}$  and then once with alcohol and once with ether. Dry at  $110^\circ$  (4 hours usually sufficient), cool, and weigh. Burn off starch, weigh again, and determine the starch by difference. (Results by this method on cream of tartar powders and tartaric acid powders agree closely with those obtained by Cu reduction. Results on other types of baking powders are usually satisfactory, but in some instances they may be over 2% too high.)

#### ALUMINUM

##### *Qualitative Test (12)—Tentative*

(In presence of phosphates)

##### 17.22

##### REAGENTS

(a) *Hydrochloric acid*.—Approximately normal. Dilute 9 ml of  $\text{HCl}$  to 100 ml.

(b) *Ammonium acetate soln*.—3 N. Dissolve 23.1 g of  $\text{NH}_4$  acetate in  $\text{H}_2\text{O}$  and dilute to 100 ml.

(c) *Aurintricarboxylic acid soln*.—0.1%. Dissolve 0.1 g in  $\text{H}_2\text{O}$  and dilute to 100 ml.

##### 17.23

##### DETECTION

Dissolve 1–5 g of the baking powder in 5 ml of the  $\text{HCl}$  and 5 ml of the  $\text{NH}_4$  acetate soln. Add 5 ml of the 0.1% soln of aurintricarboxylic acid soln, mix, and allow lake formation to take place. Make soln alkaline with  $\text{NH}_4\text{OH}$  containing a small quantity of  $(\text{NH}_4)_2\text{CO}_3$ . A bright persistent red precipitate indicates presence of Al.

*Quantitative Determination by Precipitation with Phenylhydrazine (13)—Tentative*

## 17.24

## REAGENTS

(a) *Ammonium bisulfite soln.*—Pass  $\text{SO}_2$  into cool soln of  $\text{NH}_4\text{OH}$  (1+1) until color of soln becomes distinctly yellow.

(b) *Phenylhydrazine bisulfite soln.*—To a few ml of phenylhydrazine add gradually saturated soln of  $\text{SO}_2$  until precipitate of phenylhydrazine sulfite, which at first separates out in crystals, is almost redissolved. (Unless freshly repurified, phenylhydrazine may not form a crystalline precipitate; in this case it may be purified by redistilling under a pressure of not more than 60 mm, discarding first distillate until temp. becomes constant.) If precipitate is completely dissolved, add a drop or two of phenylhydrazine until a slight precipitate is obtained. Filter soln before using. (From 5–10 ml of this soln in 100 ml of  $\text{H}_2\text{O}$  is sufficient for washing the  $\text{Al}(\text{OH})_3$  precipitate. If well stoppered, the concentrated soln will keep indefinitely.)

## 17.25

## DETERMINATION

Ignite 3 g of the baking powder at temp. not exceeding  $550^\circ$ . As soon as the C has burned off, take up residue in  $\text{HCl}$  (4+10) and boil gently to assist soln. Filter into 300 ml volumetric flask and wash with hot  $\text{H}_2\text{O}$ . Ignite insoluble residue and filter paper in Pt crucible and fuse residue with ca 2 g of  $\text{Na}_2\text{CO}_3$ . Dissolve fused mass in  $\text{H}_2\text{O}$  and  $\text{HCl}$  and transfer to volumetric flask containing original filtrate. Cool, and make to volume.

Transfer 100 ml aliquots to 400 ml beakers. Heat nearly to boiling, add  $\text{NH}_4\text{OH}$  (1+10) until slight permanent precipitate forms, then just redissolve this precipitate with a drop or two of the dilute  $\text{HCl}$ . Add dropwise with constant stirring 10 or 12 drops of saturated soln of  $\text{NH}_4\text{HSO}_3$ . Then add to hot soln sufficient phenylhydrazine to precipitate the  $\text{Al}(\text{OH})_3$  completely (1 or 2 ml; an excess colors soln yellow). If a permanent precipitate does not form at this point, add  $\text{NH}_4\text{OH}$  (1+10) carefully, dropwise, just to permanent precipitation, and then complete precipitation by adding a few more drops of phenylhydrazine. Let stand a few minutes for precipitate to settle, then filter while still warm. Wash precipitate with warm  $\text{H}_2\text{O}$ , containing 5–10 ml of the phenylhydrazine bisulfite soln in 100 ml, until washings give no test for Fe when yellow  $\text{NH}_4$  sulfide, 29.65(b), is added.

Place filter paper containing precipitate in weighed Pt crucible. Dry, char, and ignite at low temp. After filter paper has completely burned, continue ignition at bright red heat ( $850$ – $950^\circ$ ) to constant weight. Weigh quickly with cover on crucible as precipitate is very hygroscopic. A second weighing is always necessary. The precipitate consists of  $\text{Al}_2\text{O}_3$  and  $\text{AlPO}_4$ .

Fuse ignited precipitate with ca 2 g of  $\text{Na}_2\text{CO}_3$  and dissolve fusion in  $\text{HNO}_3$  (1+9). Transfer to 250 ml beaker and boil to insure that all the phosphoric acid is in ortho state. Cool. Transfer to 200 ml flask, make to volume, and determine  $\text{P}_2\text{O}_5$  in 50 ml aliquot as directed under 2.9 or 2.12. Multiply weight of  $\text{P}_2\text{O}_5$  obtained by 4 and subtract product from weight of combined precipitates obtained above. Difference = weight of  $\text{Al}_2\text{O}_3$  in 1 g of baking powder.

Weight  $\text{Al}_2\text{O}_3 \times 100$  = percentage of  $\text{Al}_2\text{O}_3$ .

Percentage of  $\text{Al}_2\text{O}_3 \times 4.749$  = percentage of  $\text{Na}_2\text{Al}_2(\text{SO}_4)_4$ .

If baking powder contains significant quantity of  $\text{SiO}_2$ , remove by evaporating the  $\text{HCl}$  soln of the powder to dryness and dehydrating at  $105^\circ$  2 hours. Add to dry mass 10 ml of  $\text{HCl}$  and 100 ml of  $\text{H}_2\text{O}$ , boil, filter off  $\text{SiO}_2$ , and proceed as directed above.

**17.26 INSOLUBLE ASH AND PREPARATION OF SOLUTION (14)—OFFICIAL**

Char 5 g of sample in Pt dish at a heat below redness (ca 500°). Boil carbonaceous mass with HCl (1+2.5), filter into 500 ml volumetric flask, and wash with hot H<sub>2</sub>O. Return residue, together with paper, to Pt dish, and burn to white ash. Boil again with the dilute HCl, filter, wash, unite two filtrates, and dilute to 500 ml. Incinerate residue after last filtration and weigh ash insoluble in acid.

**17.27 IRON AND ALUMINUM (14)—OFFICIAL**

Draw 100 ml aliquot of prepared soln, 17.26, and separate SiO<sub>2</sub> if necessary. Mix soln with 10% Na phosphate soln in excess. Add NH<sub>4</sub>OH until permanent precipitate is obtained, then HCl, dropwise, until precipitate is dissolved. Bring soln to boil and boil 2–3 min.; mix with considerable excess of 50% NH<sub>4</sub> acetate soln and 4 ml of 80% acetic acid. As soon as precipitate of AlPO<sub>4</sub>, mixed with FePO<sub>4</sub>, has settled, collect on filter, wash with hot H<sub>2</sub>O, ignite, and weigh. Fuse mixed phosphates with 10 parts of Na<sub>2</sub>CO<sub>3</sub>, dissolve in H<sub>2</sub>SO<sub>4</sub> (1+6), reduce with Zn, and determine Fe by titration with standard permanganate soln (1 ml = 1 mg of Fe). In same soln determine P<sub>2</sub>O<sub>5</sub> as directed under 2.9 or 2.12. To obtain weight of Al<sub>2</sub>O<sub>3</sub>, subtract sum of weights of Fe<sub>2</sub>O<sub>3</sub> and P<sub>2</sub>O<sub>5</sub> from weight of mixed phosphates.

**17.28 CALCIUM (14)—OFFICIAL**

Heat combined filtrate and washings obtained under 17.27 to 50° and add excess of saturated NH<sub>4</sub> oxalate soln. Allow to stand in warm place until precipitate has settled, filter, wash precipitate with hot H<sub>2</sub>O, dry, and ignite over Bunsen burner and finally over blast lamp. Cool in desiccator and weigh as CaO.

**17.29 POTASSIUM AND SODIUM (14)—OFFICIAL**

Evaporate aliquot of prepared soln, 17.26, nearly to dryness to remove excess of HCl, dilute, and heat to boiling. While still boiling add 10% BaCl<sub>2</sub> soln as long as a precipitate forms and then enough saturated Ba(OH)<sub>2</sub> soln to make liquid strongly alkaline. As soon as precipitate has settled, filter and wash with hot H<sub>2</sub>O; heat filtrate to boiling; add sufficient (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub> [1 part (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub> in 5 of NH<sub>4</sub>OH soln (1+12)] to precipitate all the Ba; filter, and wash with hot H<sub>2</sub>O. Evaporate filtrate to dryness and ignite residue below redness to remove NH<sub>4</sub> salts. Add to residue a little H<sub>2</sub>O and a few drops of the (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub> soln. Filter into weighed Pt dish, evaporate, ignite below redness, and weigh the mixed K and Na chlorides. Digest residue with hot H<sub>2</sub>O, filter thru a small filter, and dilute filtrate, if necessary, to provide for each decigram of K<sub>2</sub>O at least 20 ml of liquid. Acidify with a few drops of HCl and add an excess of Pt soln, 2.40(b). Evaporate on water bath to a thick paste; treat residue repeatedly with 80% alcohol, decanting thru weighed Gooch crucible or other form of filter, transfer precipitate to filter, and wash thoroly with the 80% alcohol. Dry 30 min. at 100° and weigh. Calculate the K so found to its equivalent of KCl and subtract result from weight of the mixed chlorides to obtain weight of NaCl.

**17.30 PHOSPHORIC ACID—OFFICIAL**

Mix 5 g of sample with a little Mg(NO<sub>3</sub>)<sub>2</sub> soln, 2.7(e), dry, ignite, dissolve in HCl (1+2.5), and dilute soln to definite volume. In an aliquot of the soln determine phosphoric acid as directed under 2.9 or 2.12.

**17.31 Qualitative Test—Tentative**

Add 10 ml of H<sub>2</sub>O to 1–2 g of baking powder in 150 ml beaker. Make just acid with HNO<sub>3</sub>, filter, take equal volumes of the filtrate and ammonium molybdate soln,

2.7(a), and warm at 40–50°. Yellow precipitate indicates presence of phosphoric acid.

## 17.32

## SULFURIC ACID (15)—OFFICIAL

Boil 5 g of sample 1.5 hours with mixture of 300 ml of H<sub>2</sub>O and 15 ml of HCl. Filter, wash filter thoroly with hot H<sub>2</sub>O, cool combined filtrate and washings, and dilute to volume of 500 ml. Determine H<sub>2</sub>SO<sub>4</sub> in an aliquot of 100 ml as directed under 12.34.

## 17.33

## AMMONIA—OFFICIAL

Introduce 2 g of sample into distillation flask, add 300–400 ml of H<sub>2</sub>O and an excess of NaOH soln (1+1), connect with condenser, and distil into measured volume of standard acid. Titrate excess of acid in distillate with standard alkali, using methyl red or cochineal indicator.

## 17.34

## ARSENIC—TENTATIVE

Introduce 5 g of sample directly into generator described under 29.2(a); add 10 ml of H<sub>2</sub>O, a little at a time to prevent foaming over, and then 15 ml of As-free HCl, introducing it dropwise until foaming ceases. Heat on steam bath until a drop of the mixture, when diluted and treated with I soln, shows no blue color. Then dilute to ca 30 ml with H<sub>2</sub>O and continue as directed under 29.5, beginning "add 5 ml of the KI reagent." Make blank and standards for comparison by use of the As-free HCl of the same concentration as that used in determination.

## 17.35

## FLUORINE—TENTATIVE.—See 29.22–29.28

## 17.36

## LEAD—TENTATIVE.—See 29.34–29.48

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## 18. COFFEE AND TEA

### GREEN COFFEE

#### 18.1 MACROSCOPIC EXAMINATION—TENTATIVE

A macroscopic examination usually shows presence of excessive quantities of black and blighted coffee beans, coffee hulls, stones, and other foreign matter. Separate these by hand picking and determine quantity gravimetrically.

#### 18.2 COLORING MATTERS—TENTATIVE

Shake vigorously 100 g or more of sample with cold  $H_2O$  or alcohol, 70% by volume. Strain thru coarse sieve and allow to settle. Identify soluble colors in soln and insoluble pigments in sediment as directed under Chap. 21.

### ROASTED COFFEE

#### 18.3 MACROSCOPIC EXAMINATION—TENTATIVE

Artificial coffee beans are apparent from their regularity of form, and roasted legumes and lumps of chicory in whole roasted coffee can be picked out and identified microscopically. For ground coffee sprinkle some of sample on cold  $H_2O$  and stir lightly. Fragments of pure coffee, if not overroasted, will float, while fragments of chicory, legumes, cereals, etc., will sink immediately, chicory coloring the  $H_2O$  a decided brown. In all cases identify particles that sink by microscopic examination.

#### 18.4 PREPARATION OF SAMPLE—OFFICIAL

Grind sample to pass thru 30-mesh sieve and preserve in tightly stoppered bottle.

#### 18.5 MOISTURE—TENTATIVE

Dry 5 g of sample at temp. of boiling  $H_2O$  under pressure not to exceed 100 mm of Hg, or at temp. of 105–110° under atmospheric pressure, for 5 hours and subsequent periods of 1 hour each until constant weight is obtained. For whole coffee, grind rapidly to coarse powder and without sifting and unnecessary exposure to air weigh portions for the determination. For ground coffee, sample directly without further grinding.

#### 18.6 SOLUBLE SOLIDS—TENTATIVE

Place 4 g of prepared sample, 18.4, in 200 ml flask. Add  $H_2O$  to mark, allow mass to infuse 8 hours, with occasional shaking, and let stand 16 hours longer without shaking. Filter, and evaporate 50 ml of filtrate to dryness in flat-bottomed dish. Dry at 100°, cool, and weigh.

#### 18.7 ASH—OFFICIAL

Proceed as directed under 34.9 or 34.10, using sample prepared as directed under 18.4.

#### 18.8 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under 34.13, using ash obtained under 18.7.

#### 18.9 ALKALINITY OF SOLUBLE ASH—OFFICIAL

Proceed as directed under 34.14, using filtrate obtained under 18.8.

**18.10**                      **ASH INSOLUBLE IN ACID—OFFICIAL**

Proceed as directed under 33.6, using ash obtained as directed under 18.7 or water-insoluble ash obtained as directed under 18.8.

**18.11**                      **SOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL**

Proceed as directed under 2.9 or 2.12, using soln obtained under 18.8.

**18.12**                      **INSOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL**

Boil insoluble ash obtained as directed under 18.8 with 25 ml of HCl (1+2), filter, wash thoroly with hot H<sub>2</sub>O, and determine P<sub>2</sub>O<sub>5</sub> in combined filtrate and washings as directed under 2.9 or 2.12.

**18.13**                      **CHLORIDES—OFFICIAL**

Proceed as directed under 12.41 and 12.42.

**CAFFEINE****18.14**                      *Power-Chesnut Method (1)—Official*

Moisten 10 g of prepared sample, 18.4, with alcohol; transfer to Soxhlet or similar extraction apparatus; and extract with alcohol 8 hours, exercising care to assure complete extraction. Transfer extract with aid of hot H<sub>2</sub>O to porcelain dish containing 10 g of heavy MgO in suspension in 100 ml of H<sub>2</sub>O. Evaporate slowly on steam bath with frequent stirring to dry, powdery mass. Rub residue with pestle into paste with boiling H<sub>2</sub>O and transfer with hot H<sub>2</sub>O to smooth filter, cleaning dish with policeman. Collect filtrate in liter flask marked at 250 ml and wash with boiling H<sub>2</sub>O until filtrate reaches mark. Add 20 ml of H<sub>2</sub>SO<sub>4</sub> (1+9) and boil gently 30 min. with a funnel in neck of flask. Cool, filter thru moistened double paper into separator, and wash with small portions of H<sub>2</sub>SO<sub>4</sub> (1+199). Extract with 6 successive 25 ml portions of CHCl<sub>3</sub>. Wash combined CHCl<sub>3</sub> extracts in separator with 5 ml of 1% KOH soln. Filter the CHCl<sub>3</sub> into Erlenmeyer flask. Wash the KOH soln with 2 portions of CHCl<sub>3</sub> of 10 ml each, adding them to flask together with the CHCl<sub>3</sub> washings of filter paper. Evaporate or distil on steam bath to small volume (10–15 ml), transfer with CHCl<sub>3</sub> to weighed beaker, evaporate carefully, dry 30 min. at 100°, and weigh. Test purity of residue by determining N and multiplying by factor 3.464.

With products very low in caffeine combine caffeine residues from duplicate determinations (representing 20 g of original material) and determine N as directed in 2.24 or 2.25, using half the quantity of reagents specified for digestion and steaming out apparatus thoroly before distilling. Distil to small volume in distilling flask to insure removal of all NH<sub>3</sub>. Correct for blank obtained, using same reagents and apparatus, and pure sucrose in place of caffeine.

**18.15**                      *Fendler-Stüber Method (Modified) (2)—Tentative*

(Adapted for quick results.)

Treat 10 g of prepared sample, 18.4, with 10 ml of NH<sub>4</sub>OH (1+2) and 200 g of CHCl<sub>3</sub> in glass-stoppered bottle; shake continuously by machine or hand 30 min., and chill in ice bath. Pour entire contents of bottle on 24 cm folded filter, covering immediately with watch-glass. Collect filtrate with funnel resting directly in neck of flask (previously weighed with stopper) and having flask surrounded with ice. Stopper as soon as soln ceases to run from funnel in a continuous stream and weigh. Evaporate on steam bath, removing last CHCl<sub>3</sub> with current of air. Digest residue

with 80 ml of hot  $\text{H}_2\text{O}$  10 min. on steam bath, shaking frequently, and let cool. Treat soln with 1%  $\text{KMnO}_4$  soln (20 ml for roasted and 10 ml for green) and let stand 15 min. at room temp., shaking occasionally. Add 2 ml of  $\text{H}_2\text{O}_2$  soln (100 ml of 3%  $\text{H}_2\text{O}_2$ , free of acetanilid, plus 1 ml of acetic acid). If liquid is still red or reddish, add the  $\text{H}_2\text{O}_2$  soln, 1 ml at a time, until excess of  $\text{KMnO}_4$  is destroyed. Place flask on steam bath 15 min. and add 0.5 ml portions of the  $\text{H}_2\text{O}_2$  soln until liquid ceases to become lighter. Cool, and filter by suction thru Gooch crucible, washing with cold  $\text{H}_2\text{O}$ . Transfer filtrate to separator and extract 6 times with 25 ml portions of  $\text{CHCl}_3$ . Evaporate combined  $\text{CHCl}_3$  extracts to small volume, transfer to weighed beaker, finish evaporation, dry at  $100^\circ$  to constant weight (30 min. is usually sufficient), and weigh residue as caffeine. Weight of caffeine  $\times 2000 \div$  weight of the  $\text{CHCl}_3$  aliquot obtained from first filtration = percentage of caffeine in the 10 g sample. Test purity of residue as directed in 18.14.

**18.16 CRUDE FIBER—OFFICIAL**

Proceed as directed under 27.30, using prepared sample, 18.4.

**18.17 STARCH—TENTATIVE**

Extract 5 g of prepared sample, 18.4, on hardened filter with 5 successive 10 ml portions of ether; wash with small portions of alcohol until total of 200 ml has passed thru; and proceed as directed under 27.35, beginning with second sentence.

**18.18 SUGARS (s)—TENTATIVE**

Weigh 10 g of prepared sample, 18.4, into 250 ml volumetric flask; add 1 g of powdered  $\text{NH}_4\text{NaHPO}_4$  and proceed as directed under 27.31 and 27.32. Determine Cu in  $\text{Cu}_2\text{O}$  precipitate either volumetrically, 34.41, or electrolytically, 34.45.

**18.19 PETROLEUM BENZINE EXTRACT—OFFICIAL**

Dry 2 g of prepared sample, 18.4, at  $100^\circ$ , extract with petroleum benzine (b.p.  $35\text{--}50^\circ$ ) 16 hours, evaporate solvent, dry residue at  $100^\circ$ , cool, and weigh.

**18.20 TOTAL ACIDITY—TENTATIVE**

Treat 10 g of prepared sample, 18.4, with 75 ml of alcohol, 80% by volume, in Erlenmeyer flask; stopper; and allow to stand 16 hours, shaking occasionally. Filter, and transfer aliquot of filtrate (25 ml for green coffee, 10 ml for roasted coffee) to beaker; dilute to ca 100 ml with  $\text{H}_2\text{O}$ ; and titrate with 0.1 *N* alkali, using phenolphthalein indicator. Express result as number of ml of 0.1 *N* alkali required to neutralize acidity of 100 g of sample.

**18.21 VOLATILE ACIDITY—TENTATIVE**

Into volatile acid apparatus, 15.24, introduce a few glass beads and over these place 20 g of the unground sample. Add 100 ml of recently boiled  $\text{H}_2\text{O}$ , place a sufficient quantity of recently boiled  $\text{H}_2\text{O}$  in outer flask, and distil until distillate is no longer acid to litmus paper (usually 100 ml of distillate will be collected). Titrate distillate with 0.1 *N* alkali, using phenolphthalein indicator. Express result as number of ml of 0.1 *N* alkali required to neutralize acidity of 100 g of sample.

**COATING AND GLAZING SUBSTANCES**

**18.22 SUGAR AND DEXTRIN—TENTATIVE**

Introduce 100 g of whole coffee into beaker, add exactly 300 ml of  $\text{H}_2\text{O}$ , stir, and allow to stand 5 min., stirring frequently. Filter thru dry filter and add carefully to

filtrate dry Pb acetate until precipitation is complete, avoiding excess of the reagent. Filter thru dry filter and remove Pb from filtrate by addition of slight excess of dry, powdered K oxalate. Filter thru dry filter and determine reducing sugars as invert sugar in 50 ml of the filtrate, as directed under 34.39. Invert 75 ml aliquot of filtrate as directed under 34.24(b). Cool, nearly neutralize with NaOH soln (1+1), dilute to 100 ml, and determine reducing sugars as invert sugar in resulting soln as directed under 34.39. Measure 100 ml aliquot of filtrate into 200 ml flask, add 10 ml of HCl (sp. gr. 1.125), and hydrolyze as directed under 27.33. Cool, neutralize with NaOH soln (1+1), dilute to volume, filter thru dry filter, and determine reducing sugars as invert sugar in 50 ml of filtrate as directed under 34.39. Calculate reducing sugars in each instance to percentage by weight of original coffee. Calculate sucrose from reducing sugars before and after inversion as directed under 34.30, and calculate dextrin as follows: Subtract reducing sugars after inversion from reducing sugars after hydrolysis and multiply difference by factor 0.8605 to convert result to dextrin.

In some instances presence of sucrose in the water extract may be verified by polarization. Presence of dextrin in the water extract may be verified by polarization as directed under 34.32, and by erythrodextrin test, 34.100, made on water extract previous to clarification with Pb acetate.

#### 18.23 EGG ALBUMIN AND GELATIN—TENTATIVE

Treat 100 g of whole coffee with 500 ml of H<sub>2</sub>O and allow to stand 5 min., stirring frequently. Filter, and treat separate portions of filtrate with (1) 5% soln of tannic acid, and (2) Millon reagent, 27.13. Boil a third portion of filtrate. In the presence of egg albumin a more or less heavy precipitate will be formed in each case. As a confirmatory test, treat aliquot of filtrate with excess of tannic acid soln; add a little NaCl if necessary to secure flocculation of precipitate; filter; and, without washing, introduce paper and its contents into Kjeldahl flask and determine N. By this method coffee not coated with albumin or gelatin will yield less than 10 mg of N/100 g of sample.

#### 18.24 CHICORY INFUSION—TENTATIVE

Cover 100–150 g of whole coffee with H<sub>2</sub>O; allow to soak 2–3 min., stirring frequently; and drain aqueous washings thru coarse sieve. Wash coffee upon sieve with ca 100 ml of H<sub>2</sub>O and centrifuge combined washings. Decant clear liquid from sediment, which should then be drained almost dry on filter paper. Mount sediment in chloral hydrate soln, 33.30(c), and examine under microscope for elements of chicory.

#### 18.25 FATS AND WAXES (4)—TENTATIVE

Treat 100–200 g of the beans with low-boiling petroleum benzine 10 min., pour off petroleum benzine, and repeat process. Filter combined extract, evaporate, and determine index of refraction and saponification number of residue as directed under 31.9 and 31.25.

### TEA

#### 18.26 DUST, STEMS, AND FOREIGN LEAVES—TENTATIVE

Place 1 g of the tea in 300 ml casserole, add 200 ml of boiling H<sub>2</sub>O and allow to stand 15 min. This treatment will cause the leaves to unroll, and they will then be in condition for examination as to form and structure (5). A macroscopic examination will reveal presence or absence of dust or stems. Only those stems that remain



floating after the leaf is thoroly infused should be regarded as woody stems (6) ("floaters").

**18.27 PREPARATION OF SAMPLE—OFFICIAL**

Grind the sample to pass thru 30-mesh sieve.

**18.28 MOISTURE—OFFICIAL.—See 27.3**

**18.29 WATER EXTRACT (7)—OFFICIAL**

To 2 g of the ground sample in 500 ml volumetric flask, add 200 ml of hot H<sub>2</sub>O and boil over low flame 1 hour, rotating occasionally. Close flask with rubber stopper thru which passes glass tube 30" long for condenser. Boil very slowly so that no steam escapes from top of air condenser. Cool, dilute to volume, mix thoroly, and filter thru dry filter paper. Transfer 50 ml aliquot to weighed dish and evaporate to dryness on steam bath. Place in oven, heat at 100° 1 hour, cool, and weigh.

**18.30 ASH—OFFICIAL.—See 34.9 or 34.10**

**18.31 SOLUBLE AND INSOLUBLE ASH—OFFICIAL**

Proceed as directed under 34.13, using ash obtained under 18.30.

**18.32 ALKALINITY OF SOLUBLE ASH—OFFICIAL**

Proceed as directed under 34.14, using filtrate obtained under 18.31.

**18.33 ALKALINITY OF INSOLUBLE ASH—OFFICIAL**

Proceed as directed under 34.15, using insoluble ash obtained under 18.31.

**18.34 ASH INSOLUBLE IN ACID—OFFICIAL**

Proceed as directed under 33.6, using total ash obtained as directed under 18.30, or insoluble residue obtained under 18.31.

**18.35 SOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL**

Proceed as directed under 2.9 or 2.12, using soln of soluble ash obtained under 18.32.

**18.36 INSOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL**

Proceed as directed under 2.9 or 2.12, using soln obtained under 18.33.

**18.37 PETROLEUM BENZINE EXTRACT—OFFICIAL.—See 18.19**

**18.38 PROTEIN—TENTATIVE**

Determine N as directed under 2.24, 2.25, or 2.26. To obtain percentage of N present as protein, subtract percentage of N present as caffeine from percentage of total N. Multiply this result by 6.25 to obtain percentage of protein.

**18.39 CRUDE FIBER—OFFICIAL.—See 27.30**

**CAFFEINE**

**18.40 Power-Chesnut Method (8)—Official**

Proceed as directed under 18.14.

**18.41 Bailey-Andrew Method (9)—Official**

To 5 g of prepared sample, 18.27, in 500 ml volumetric flask, add 10 g of heavy MgO and 200 ml of H<sub>2</sub>O. Boil gently over low flame 2 hours, using small-bore glass

tube 30" long as condenser. Cool, dilute to volume, and filter thru dry paper. Transfer aliquot portion of 300 ml, equivalent to 3 g of original material, to Erlenmeyer flask of 1 liter capacity; add 10 ml of  $H_2SO_4$  (1+9); and boil until volume is reduced to ca 100 ml. Filter into separator, washing flask with small portions of  $H_2SO_4$  (1+99), and shake 6 times with  $CHCl_3$ , using 25, 20, 15, 10, 10, 10 ml portions. Treat combined extracts with 5 ml of 1% KOH soln and when liquids have completely separated draw off  $CHCl_3$  layer into suitable flask or beaker. Wash the alkaline soln in separator with 2 portions of  $CHCl_3$ , of 10 ml each, and unite washings with main bulk of extract. Evaporate or distil off the  $CHCl_3$  to small bulk, transfer to weighed flask, evaporate to dryness, and further dry in oven at 100° to constant weight. Test purity of residue by determining N and multiplying by factor 3.464. This gives a value for anhydrous caffeine.

#### TANNIN (10)—TENTATIVE

##### 18.42

##### REAGENTS

(a) *Potassium permanganate soln.*—Prepare soln containing 1.33 g/liter and obtain its equivalent in terms of 0.1 N oxalic acid.

(b) *Indigo carmine soln.*—Prepare soln containing 6 g of indigo carmine (free from indigo blue) and 50 ml of  $H_2SO_4$ /liter.

(c) *Gelatin soln.*—Soak 25 g of gelatin 1 hour in saturated NaCl soln, heat until gelatin is dissolved, cool, and dilute with saturated NaCl soln to 1 liter.

(d) *Acid sodium chloride soln.*—Acidify 975 ml of saturated NaCl soln with 25 ml of  $H_2SO_4$ .

##### 18.43

##### DETERMINATION

Boil 5 g of the tea 30 min. with 400 ml of  $H_2O$ , cool, transfer to 500 ml volumetric flask, and dilute to mark. To 10 ml of the infusion (filtered, if not clear) add 25 ml of the indigo carmine soln and ca 750 ml of  $H_2O$ . Add the  $KMnO_4$  soln from a buret, a little at a time while stirring, until color becomes light green, then dropwise until color changes to bright yellow or to faint pink at rim. Designate number of ml of  $KMnO_4$  used as "a."

Mix 100 ml of the clear infusion of tea with 50 ml of the gelatin soln, 100 ml of the acid NaCl soln, and 10 g of powdered kaolin, and shake several minutes in stoppered flask. After allowing mixture to settle, decant thru filter. Mix 25 ml of filtrate with 25 ml of the indigo carmine soln and ca 750 ml of  $H_2O$  and titrate with  $KMnO_4$  as before. Number of ml of  $KMnO_4$  used subtracted from that obtained above, "a," gives quantity of  $KMnO_4$  required to oxidize the tannin. 1 ml of 0.1 N oxalic acid = ca 0.0042 g of tannin (gallotannic acid).

#### FACING

##### 18.44

##### GENERAL (11)—TENTATIVE

(1) Examine ash obtained as directed under 18.30 for mineral pigments, 21.1; (2) shake quantity of the tea with large volume of  $H_2O$  and remove leaves by means of sieve. Allow insoluble matter in the  $H_2O$  portion to settle, filter, and examine residue on filter paper for insoluble pigments as directed under 21.1. Catechu and other soluble substances, if used, will be found in filtrate.

##### 18.45

##### PARAFFIN AND WAXY SUBSTANCES—TENTATIVE

Spread a quantity of the tea between two sheets of unglazed white paper and place thereon a hot iron. Any greasy substance will stain the paper (18).

**18.46      PIGMENTS USED FOR COLORING OR FACING (18)—TENTATIVE**

Place 60 g of the tea in 60-mesh, 5–6" sieve provided with top. Sift small quantity (ca 0.1 g) of the dust upon piece of semi-glazed, white paper ca  $8 \times 10"$ . (To obtain requisite quantity of dust, it is sometimes necessary to rub leaf gently against bottom of sieve.) Place paper on plain, firm surface, preferably glass or marble, and crush dust by pressing firmly upon it flat steel spatula ca 5" long. Repeat crushing process until tea dust is ground almost to powder, when particles of coloring matter, if present, become visible as streaks on the paper. Brush off loose dust and examine paper by means of simple lens magnifying 7.5 diameters. Bright light is essential to distinguish these particles and streaks. In many cases the character of the pigment is indicated by the behavior of these streaks when treated with reagents and examined under a microscope. The crushed particles of leaf of either black or green tea appear in such quantity that there is no chance of mistaking them for coloring or facing material. Repeat this test, using black, semi-glazed paper for facings such as talc, gypsum,  $\text{BaSO}_4$ , or clay.

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- (10) U. S. Dept. Agr. Div. Chem. Bull. **13** (VII), p. 890.
- (11) U. S. Dept. Agr. Bur. Chem. Bull. **90**, p. 38.
- (12) U. S. Treas. Dept. T. D. 35244, March 23, 1915; U. S. Dept. Agr. Misc. Circ. **9**, Reg. 23.
- (13) U. S. Treas. Dept. T. D. 35244, March 23, 1915; Proc. Eighth Intern. Cong. Appl. Chem., **18**, 301 (1912); U. S. Dept. Agr. Misc. Circ. **9**, Reg. 24.

## 19. CACAO BEAN AND ITS PRODUCTS

### 19.1

#### PREPARATION OF SAMPLE

(a) *Official*.—Mix powdered products thoroly and preserve in tightly stoppered bottles. Chill sweet or bitter chocolate until it becomes hard and reduce to fine granular condition by grating or shaving. Mix thoroly and preserve in tightly stoppered bottle in cool place.

(b) *Tentative*.—Melt bitter, sweet, or milk chocolate by placing in glass or metal container and partly immersing container in bath at ca 50°. Stir frequently until sample melts and attains a temp. of 45–50°. Remove from bath, stir thoroly, and remove a portion of suitable size for analysis while in liquid condition, using glass or metal tube with diameter of 4–10 mm, provided with close-fitting plunger to expel sample from tube.

### 19.2

#### MOISTURE—TENTATIVE

Dry 2 g of prepared sample, 19.1, to constant weight in Pt dish in air oven at 100°. (Al dish may be used when ash is not determined on same sample.) Report loss in weight as moisture.

### 19.3

#### ASH—OFFICIAL

Proceed as directed under 34.9 or 34.10, using sufficient sample to contain ca 1 g of moisture-, sugar-, and fat-free material.

### 19.4

#### SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under 34.13, using ash obtained under 19.3.

### 19.5

#### ALKALINITY OF SOLUBLE ASH—OFFICIAL

Proceed as directed under 34.14, using filtrate from 19.4.

### 19.6

#### ALKALINITY OF INSOLUBLE ASH—OFFICIAL

Proceed as directed under 34.15, using insoluble ash obtained under 19.4.

### 19.7

#### ASH INSOLUBLE IN ACID—OFFICIAL

Proceed as directed under 33.6, using total ash obtained under 19.3, or water-insoluble residue obtained under 19.4.

### 19.8

#### TOTAL NITROGEN—OFFICIAL.—See 2.24, 2.25, or 2.26

### 19.9

#### COLORING MATTERS—TENTATIVE.—See 21.2(e)

### SHELL (1)

#### *In Cacao Nibs—Tentative*

### 19.10

#### TRIER FOR SAMPLING

Use a double-tube, separate-compartment grain trier to collect samples of nibs from bins, trucks, and sacks. Tubes of trier are of No. 16 gage (B & S) (0.0508") seamless metal. Outside diam. of outer tube is 1½", and outer and inner tubes fit each other closely. Width of openings in outer tube is 15/16", and in inner tube 1".

Length of such openings in both tubes is  $3\frac{1}{2}$ ", except that length of opening of compartment nearest point of trier may be  $3-3\frac{1}{2}$ ". Each compartment coincides with and is of same length as its opening in the inner tube. Openings of inner and outer tubes match when trier is open for sampling. Distance between adjacent compartments is  $1\frac{1}{2}-2$ ", and distance between point of trier and compartment end nearest point is not more than  $1\frac{1}{2}$ ".

## 19.11

## COLLECTION OF SAMPLE

(a) *From bins or trucks*.—Collect sample of ca 10 lbs. by probing with the trier, 19.10. Probe nibs to floor of bin or truck, spacing individual probings ca equidistant from each other thruout top area of nibs. If contents of bin are inaccessible, or are of greater depth than length of trier from its point to 2" above compartment end nearest handle, take sample from chute thru which bin is being filled or emptied as directed in (c).

(b) *From sacks*.—Collect sample of ca 10 lbs. by probing with the trier, 19.10. Length of trier from its point to 2" above compartment end nearest handle equals or exceeds depth to which sacks are filled. Probe with trier thru entire depth of nibs in sack. Probe a number of sacks equal to at least the square root of total number of sacks in lot. If lot is of less than 12 sacks, probe at least  $\frac{2}{3}$  of them; if of more than 12, probe at least 8.

(c) *From chutes*.—Collect sample of ca 10 lbs. by catching momentarily and at regular intervals in suitable receptacle a cross section of the stream of nibs from chute. Continue sampling thruout time the lot of nibs being sampled is passing thru chute.

## 19.12

## REDUCTION OF SAMPLE

Using sample divider of type described in U. S. Department of Agriculture Bull. No. 287, September 14, 1915, and No. 857, June 25, 1920, reduce size of sample collected, 19.11, to ca  $\frac{1}{4}$  lb. Weigh reduced sample to nearest 0.05 g.

## 19.13

## DIVISION OF SAMPLE

(a) *Hand division*.—Screen reduced sample, 19.12, in successive portions of 75–100 g, on circular sieve, bottom of which is No. 10 woven wire cloth that complies with specifications for such cloth set forth on page 3 of the publication, "Standard Specifications for Sieves," October 25, 1938, U. S. Department of Commerce, National Bureau of Standards. Diameter of sieve is ca 8". Collect material remaining on sieve and designate it as L. Screen material that passed thru sieve on another circular sieve, bottom of which is No. 20 wire cloth that complies with specifications for such cloth set forth on the same page of such publication. Diameter of sieve is either 6 or 8". Collect portion remaining on sieve and designate it as S. Collect material passing thru sieve and designate it as F. Treat portions L, S, and F, respectively, as directed under 19.14(a), (b), and (c).

(b) *Machine division*.—Use a sample-size, grain-cleaning mill of type described on page 16 of U. S. Department of Agriculture Farmers' Bull. 1747 (1935). Fit into lower slot of mill a single screen having circular openings 0.083–0.093" in diam. The machine is provided with settling traps to catch all material blown out by fan. Inclined slide under screen is provided with removable slat in such position that when it is removed the material passing thru screen is discharged from mill without going to fanning chamber. Remove this slat, start mill, and slowly pour reduced sample, 19.12, over upper part of screen. Designate as L' the material that does not

pass thru screen and is not removed by fanning. Collect material that passes thru screen and screen it again on sieve having No. 20 woven wire cloth (a). Collect portion remaining on sieve and designate it as S. Collect portion passing thru sieve and designate it as F. Reserve F for treatment as directed in 19.14(c).

Replace slat in mill and without removing L' or fannings, start mill and pour S slowly onto upper part of screen. Allow material thus cleaned to combine with L', and designate combination as L' S'. Treat L' S' as directed in 19.14(a). Remove combined fannings from settling traps and screen on sieve having No. 10 wire cloth, (a). Collect portion remaining on sieve and designate it as LS. Collect portion passing thru sieve and designate it as SS. Treat LS as directed in 19.14(a), and SS as directed in 19.14(b).

### 19.14

#### DETERMINATION

(a) Place L (from hand division), or L' S' (from machine division), on large sheet of sized paper. Scatter 2-3 g of L or 4-5 g of L' S' over an area of the paper ca 3-4" in diam. Examine scattered portion and remove pieces of shell with spatula or tweezers. Examine entire portion of L or L' S' progressively in this manner. Separate shell from LS (from machine division) in same manner. Reserve separated shell for later combination with shell from other fractions.

(b) If S (from hand division) weighs more than 4.5 g and appears to contain large quantity of shell, weigh it accurately to nearest 10 mg; mix entire portion by pouring it gradually several times from one glazed paper to another, each time forming conical piles; flatten and quarter last pile formed, and combine alternate quarters; if necessary mix and again reduce by quartering until a weight of 3-4.5 g is obtained; and weigh fraction thus obtained accurately to nearest 10 mg. If quantity of shell in S appears to be small, use entire portion. Separate shell from S or fraction thereof and from SS (from machine division) as follows:

Place a blotting paper ca 19×24" on firm supporting plane inclined at an angle of 21-24° from horizontal. Pour all the material gradually and in successive portions from an elevation of 2-3" along upper end of blotter. Shake blotter slowly parallel to plane to cause nib material to roll down, and at intervals remove material collected at bottom. Toward end of procedure shake blotter more rapidly to detach most of nib material. After removing shell adhering to blotter repeat procedure on last portions of material collected at bottom of blotter. Using a reading glass, complete separation of shell and nibs with spatula or tweezers by examining portions until all material is examined. Except in separations from a fraction of S, reserve separated shell for combination with that obtained from other separations. In the case of separations from a fraction of S, weigh separated shell to nearest mg and calculate total weight of shell in S.

(c) Place F (obtained from either hand division or machine division) in 400 ml beaker about half full of a soln composed of 2½ volumes of CCl<sub>4</sub> and 1 volume of alcohol. (Soln should have sp. gr. of 1.335-1.345 at temp. used as compared to H<sub>2</sub>O at 20°.) Stir mixture ca 1 min., but slowly at the last, and allow to stand 3-4 min. Skim off floating nibs with tea strainer made with ca No. 40 wire cloth. Decant liquid and any suspended material from beaker without disturbing residue until 2-4 ml remains. Wipe inside of beaker above liquid with filter paper, moistened in the soln described above, so as to remove all nib material. Add ca 25 ml of petroleum benzine, swirl liquid in beaker a few times, allow residue of shell to settle, and carefully decant liquid. Allow remaining liquid to evaporate and dry the shell on steam bath. Reserve shell for combination with other fractions of shell.

(d) Combine all shell obtained from L, S, and F (from hand division) or L' S',

LS, SS, and F (from machine division) and weigh combined shell from reduced sample. If a fraction of S was used, combine L and F, weigh, and add calculated weight of shell in S to obtain weight of shell from reduced sample. Report result in terms of percentage by weight of shell in nibs.

*In Cacao Products Other Than Cacao Nibs*

(The following methods include determinations results of which can be used to estimate amount of shell when compared with corresponding values obtained on authentic samples of cacao shell.)

19.15

CRUDE FIBER (S)—OFFICIAL

(a) *Applicable to cacao products not containing dairy ingredients.*—Treat 7 g of liquor (or quantity of sweet chocolate or cocoa equivalent to 7 g of liquor) in nursing bottle with two 100 ml portions of ether, centrifuging and decanting supernatant liquor after each addition. Dry residue in oven at ca 100° and then powder in bottle with flattened glass rod. (In some cases it may be necessary to grind material in mortar and extract third time with ether.) Wash mixture in nursing bottle with three 100 ml portions of H<sub>2</sub>O at room temp., shaking well each time, until no cacao material adheres to bottle. Centrifuge after each washing 10–15 min., and decant aqueous layer. Wash residue in same fashion with two 100 ml portions of alcohol and one 100 ml portion of ether. Transfer residue to Pt dish, dry to constant weight, and grind in mortar. Weigh 2 g of dried material and determine percentage of crude fiber (*D*) as directed under 27.30, using linen for both acid and alkaline filtrations. Calculate percentage of crude fiber on moisture-, fat- and sugar-free basis (*E*) by the formula  $E = 0.7D$ .

(b) *Applicable to cacao products containing dairy ingredients.*—Treat 50 g of milk chocolate with three 100 ml portions of ether in nursing bottle, centrifuging and decanting supernatant liquor after each addition. Dry residue in the bottle and powder with flattened glass rod. Shake with 100 ml of 1% Na<sub>2</sub>C<sub>2</sub>O<sub>4</sub> soln, and let stand 30 min. Centrifuge and decant supernatant liquor. Wash in nursing bottle with three 100 ml portions of H<sub>2</sub>O at room temp., shaking well each time, until no cacao material adheres to bottle. Centrifuge after each washing 10–15 min. and decant aqueous layer. Wash residue in same fashion with two 100 ml portions of alcohol and one 100 ml portion of ether. Transfer residue to Pt dish, dry to constant weight at 100°, and grind in mortar. Weigh 2 g of the dried material and determine percentage of crude fiber as directed in 27.30, using linen for both acid and alkaline filtrations. Percentage of crude fiber found  $\times 0.7$  = percentage of crude fiber on true fat-, sugar-, moisture- and milk-free basis.

19.16

PECTIC ACID (S)—TENTATIVE

(a) *Applicable to products containing no milk solids.*—Place sufficient sample to contain ca 5 g of fat-free cacao (6–8 g of cocoa, 10–12 g of liquor, or 13–80 g of sweet chocolate) in one or two centrifuge bottles according to size of sample. To remove fat, shake material thoroly with two 100 ml portions of petroleum benzine or ether, centrifuge, and decant supernatant liquid after each addition. Repeat extraction with 100 ml of alcohol and discard extract. Add to residue in each bottle 10 ml of alcohol and 5 ml of HCl (1+1) and stir with glass rod to smooth paste. Place thermometer in bottle and heat on steam bath, stirring frequently, especially around sides of bottle, until temp. reaches 60° (not above 65°). Remove from bath, rinse and remove thermometer, and gradually add 100 ml of alcohol with stirring. Rinse off rod with alcohol, stopper bottle, shake vigorously 2 min., centrifuge 6–8 min., and

decant and discard supernatant liquid. Again shake with 100 ml of alcohol, centrifuge, decant, and discard the alcohol.

Disperse the residue thoroly in 70 ml of  $H_2O$  by shaking the stoppered bottle vigorously (if in 2 bottles, shake in one and decant into other and shake again), and decant mixture into 500 ml wide-mouthed Erlenmeyer flask. Rinse bottle lip with spurt of  $H_2O$  from wash bottle. Complete transfer by shaking successively with 45 and 25 ml portions of  $H_2O$ , respectively. Neutralize to litmus with  $NH_4OH$  (1+1) (ca 1 ml), and then make slightly acid with acetic acid. Add 50 ml of 2%  $NH_4$  oxalate soln, place rod in flask reaching just below mouth, and cover with watch-glass. Place flask in bath at 90–92° (steam bath with overflow pipe inserted and filled to necessary level can be regulated closely enough) and digest for 3 hours, stirring occasionally with rod. Replace cover-glass between stirrings.

Remove flask from bath, evaporate contents to 175 ml if necessary, and cool to room temp. Transfer contents to 200 ml volumetric flask, break foam with 1–2 drops of n-hexyl alcohol, make to mark, and add 1.5 ml of  $H_2O$  for volume of cacao solids. Mix, and pour contents into centrifuge bottle (250 ml or larger). Centrifuge at ca 1800 r.p.m. for 12–15 min. Measure 175 ml of supernatant liquid into graduated cylinder and transfer to another centrifuge bottle, reserving cacao residue for further treatment. Cool bottle and liquid contents to ca 20° (below 25°), add 10 ml of 15%  $NaOH$  soln with stirring, stopper bottle, and allow to stand 30 min. at ca 20°. Add with stirring 10 ml of  $HCl$  and then stir in 30 ml of alcohol. Add 1.5 g of filter-cel, stopper, shake well, and centrifuge for ca 10 min. Decant and discard supernatant liquid without disturbing sediment containing pectic acid. Shake sediment with 100–120 ml of  $H_2O$ , make alkaline with  $NH_4OH$  (1+1), adding 1 ml in excess, and again shake thoroly to dissolve precipitates. Make acid with acetic acid, add 1 ml excess, and add slowly with stirring 5 ml of 10% tannic acid soln. Stopper bottle and shake thoroly ca 1 min. Filter on Büchner funnel (7–11 cm) with Whatman No. 54 or 41 H filter paper, using moderate suction. Wash residue twice with 20 ml portions of  $H_2O$ .

Transfer filtrate to 400 ml beaker and evaporate on hot plate to ca 65 ml. Add gradually with stirring 150 ml of alcohol, and allow to stand few minutes. Filter off precipitate on 15 cm filter paper and wash twice with alcohol. Wash precipitate back into beaker, dilute to ca 50 ml, cool to ca 20°, and repeat saponification with 3 ml of 15%  $NaOH$  soln. Dilute to ca 90 ml and precipitate pectic acid with 10 ml of 15%  $NaOH$  soln. Dilute to ca 90 ml and precipitate pectic acid with 10 ml of  $HCl$  (1+2.5). Boil 3 min., filter, and wash with  $H_2O$  until washings show negligible acidity. Wash the pectic acid into Pt dish and dry on steam bath and finally at 100° to constant weight. Weigh, ignite, and weigh again. Loss in weight is pectic acid in aliquot. This weight  $\times 1.14$  = weight of pectic acid in sample.

Transfer any cacao residue in the 200 ml volumetric flask to centrifuge bottle containing bulk of residue reserved above with 100 ml of alcohol. Stopper bottle, shake thoroly, centrifuge, decant, and discard supernatant liquid. Repeat extraction in same manner with another 100 ml portion of alcohol and once with 80 ml of ether. Allow ether to evaporate and transfer residue quantitatively to tared covered aluminum dish. Dry in oven, cover, cool, and weigh. Weight of residue  $\times 2$  = original fat-free dry cacao. Weight of pectic acid  $\div$  weight of fat-free dry cacao  $\times 100$  = percent pectic acid.

(b) *Applicable to products containing milk solids.*—Place a sample sufficient to contain an estimated 5 g of dry fat-free cacao (30–110 g, depending on liquor content) in one or two centrifuge bottles. If 250 ml bottles are used and sample exceeds 55 g use two bottles.

Remove fat by shaking with 100 ml of petroleum benzine or ether, centrifuging



and decanting. Repeat extraction twice. Place bottle in warm place and draw a gentle current of air thru it to expel the ether (15–20 min. usually sufficient).

Pulverize contents with glass rod. Add 140 ml of  $H_2O$  to each bottle and shake vigorously for several minutes. Immediately add 50 ml of 2%  $NH_4$  oxalate soln and again shake vigorously ca 2 min. Centrifuge 12–15 min. at 1800 r.p.m. and decant supernatant liquid into 600 ml beaker. Reserve this liquid for later treatment. Treat cacao residue in centrifuge bottle (or bottles) as directed in (a), beginning "Repeat extraction with 100 ml of alcohol."

To liquid in beaker reserved above, add  $HCl$  (1+5) very slowly (2 or 3 seconds between drops), with vigorous stirring, until a fine precipitate forms and does not dissolve, and the liquid lightens in color (3.5–4.2 ml will be required). Avoid use of sufficient acid to produce a heavy flocculent precipitate. Should this occur, add sufficient 15%  $NaOH$  soln to redissolve precipitate and again carefully acidify to point described above. Let stand 5 min. and place covered beaker on steam bath. Stir frequently for first 15 min. and then at intervals, allowing material to remain on bath 60–70 min., then remove. Pulp three or four 15 cm circles of Whatman No. 1 filter paper by tearing to bits and shaking with  $H_2O$ . Overlay an 11 cm Whatman 54 or 41 H paper with about two-thirds of the paper pulp in a Büchner funnel. Stir remainder of pulp into mixture in beaker and filter contents on Büchner funnel with suction. Allow to suck "dry" and wash once with ca 20 ml of  $H_2O$ . (Filtrate should be clear.) Transfer filtrate to 800 ml beaker, add 5 ml of 25%  $CCl_3COOH$  soln, and evaporate on hot plate to ca 175 ml. If insoluble matter separates, filter again and proceed with evaporation.

Cool, and transfer liquid to 200 ml volumetric flask and make to mark. Mix, and measure 175 ml (graduate) into 250 ml centrifuge bottle. Cool, and saponify pectin as directed in (a). Saponify extract of cacao residue described above in another centrifuge bottle, add only 1 g of Filter Cel to each bottle, and after centrifuging down the pectic acid, shake with  $H_2O$  and combine the two portions of pectic acid. Proceed as directed in (a), par 3, beginning "Shake sediment with 100–120 ml."

19.17

ASH INSOLUBLE IN ACID—OFFICIAL.—See 19.7

## CACAO PRODUCTS PROCESSED WITH ALKALIES

19.18

ASH—OFFICIAL.—See 19.3

19.19

SOLUBLE AND INSOLUBLE ASH—OFFICIAL.—See 19.4

19.20

ALKALINITY OF SOLUBLE ASH—OFFICIAL.—See 19.5

19.21

ALKALINITY OF INSOLUBLE ASH—OFFICIAL.—See 19.6

19.22

## CHOCOLATE LIQUOR (4)—TENTATIVE

Extract 25–50 g (50 g if light color, indicating low liquor) of sample as directed in 19.15, except to use in the first aqueous extraction 200 ml of  $H_2O$  or 1%  $Na_2C_2O_4$  soln, as the case may be.

With aid of small portions of ether (45, 20, 15 ml, etc.) transfer residue resulting from ether, alcohol, and aqueous extractions to tared Al dish provided with close-fitting cover. Use small quantity of acetone and a policeman to transfer any material that sticks to bottle. Evaporate liquid and dry residue in oven at 100°. Cover dish, cool in desiccator, and weigh.

To obtain the weight of fat-free dry cacao mass, multiply weight of residue by factor 1.43. To obtain weight of chocolate liquor multiply weight of fat-free dry cacao mass by the factor 2.2. (This factor is based on fat content of 54% in chocolate liquors.)

## FAT

### QUANTITATIVE DETERMINATION

#### 19.23

##### *Method I (5)—Official*

Prepare in Knorr extraction tube a tightly packed mat of asbestos purified as for determination of crude fiber, 27.28(c), and carefully freed from coarse pieces. Wash filter with alcohol, ether, and a little petroleum benzine. (All petroleum benzine used in this determination must be redistilled below 60°.) Weigh 2–3 g of prepared sample, 19.1, into tube and insert tube into rubber stopper in filtering bell-jar connected to suction thru two-way stopcock, taking care that no rubber particles adhere to tip of stem. Place weighed 150 ml Erlenmeyer flask at such height that the tube stem passes thru neck into flask. (Stem of tube should be lengthened if necessary.) Fill tube to ca  $\frac{3}{4}$  of capacity with the redistilled petroleum benzine, and by means of rod having flattened end stir sample thoroly, taking care to crush all lumps. Let stand 1 min. and drain by suction. Regulate suction so that collected solvent in flask will not boil violently. Add solvent from wash-bottle, at same time turning tube between thumb and finger so that sides of tube are washed down by each addition. Repeat extractions, with stirring, until fat is removed (usually 10 extractions). Remove tube with stopper from bell-jar, wash traces of fat from end of stem with petroleum benzine, evaporate solvent, and dry to constant weight at 100°.

#### 19.24

##### *Method II (6)—Tentative*

Weigh accurately ca 2 g of prepared sample, 19.1, and without previous drying stratify charge in extraction tube with ca 0.5 g of asbestos, 27.28(c), further washed with alcohol, ether, and petroleum benzine. Extract with petroleum benzine, redistilled below 60°, in continuous extractor 4 hours. Grind material to break up any lumps that may have formed and re-extract at least 4 hours. (It is advisable to allow solvent to run thru material once completely before applying heat for continuous extraction.) Collect petroleum benzine extract in weighed flask, evaporate solvent, and dry residue to constant weight at 100°.

#### 19.25

##### SEPARATION AND PREPARATION OF FAT—TENTATIVE

Separate fat from 10–40 g of sample (depending upon fat content) by shaking material with 2 or 3 100 ml portions of ether. Centrifuge and decant each portion. Combine portions in beaker and drive off most of ether on steam bath. Filter ether extracts thru dry, folded filter and dry at 100°.

#### 19.26

##### IODINE ABSORPTION NUMBER—OFFICIAL.—See 31.19 or 31.21

#### 19.27

##### MELTING POINT—OFFICIAL

Proceed as directed under 31.14. Keep fat at least 24 hours in cool place before making determination.

#### 19.28

##### INDEX OF REFRACTION—OFFICIAL.—See 31.9 or 31.11

#### 19.29

##### REICHERT-MEISSL AND POLENSKE VALUES (7)—OFFICIAL.—See 31.29

## 19.30

## MILK FAT IN MILK CHOCOLATE—TENTATIVE

Estimate quantity of milk fat in milk chocolate from following formula:

$$C = \frac{AX + BY}{5}, \quad \text{in which}$$

$A$  = g of butter fat in 5 g of mixed fat;  $B = 5 - A$  = g of cacao fat in 5 g of mixed fat;  $C$  = Reichert-Meissl number of extracted fat;  $X$  = Reichert-Meissl number of authentic butter fat; and  $Y$  = Reichert-Meissl number of authentic cacao butter.

Then weight of butterfat,  $A$ , in 5 g of mixed fat =  $\frac{5(C - Y)}{X - Y}$ , and

Percentage of butterfat = percentage of total fat  $\times \frac{C - Y}{X - Y}$ .

## 19.31

## SAPONIFICATION NUMBER—OFFICIAL.—See 31.25

## 19.32 DETECTION OF COCONUT AND PALM KERNEL OILS IN CACAO BUTTER AND FAT EXTRACTED FROM MILK CHOCOLATE (8)—TENTATIVE

(a) *Examination of cacao butter.*—Saponify 5 g of sample with 15 ml of alcoholic KOH soln (25 g to 200 ml of alcohol) and evaporate alcohol on steam bath. Run blank on pure cacao butter at same time. Add 5 ml of  $H_2O$  and again evaporate to remove last trace of alcohol. Dissolve soap in 100 ml of  $H_2O$ , cool to room temp., and add, while stirring, 100 ml of saturated NaCl soln. Allow to stand 15 min., stirring several times during this period, and then separate the soap by filtration, using Büchner funnel. To 100 ml of filtrate add, while stirring, 100 ml of the saturated NaCl soln and allow to stand 15 min. (Only a slight precipitate should appear.) Filter, add to filtrate a drop of phenolphthalein indicator, neutralize with HCl (1+3), and add 0.5 ml of this reagent in excess. If sample consists of pure cacao butter, soln when acidified will remain clear; if coconut or palm kernel oil is present, soln will become turbid or milky.

(b) *Examination of fat extracted from milk chocolate (9).*—Milk fat, if present in cacao butter subjected to this test, produces a turbidity less in intensity than that produced by same percentage of coconut or palm kernel oil. For example, cacao butter containing 10, 15, or 20% of milk fat produces, respectively, no opalescence, faint opalescence, or an opalescence. For this reason, when the fat to be examined has been extracted from a cacao product that contains lactose or casein, multiply percentage of lactose in cacao product by 0.8, or percentage of casein by 1.1, to obtain percentage of milk fat in the product, and from this result calculate percentage of milk fat in total fat. If this percentage corresponds to 15% or less, a blank of cacao butter containing 15% milk fat may be used; otherwise make up mixture of cacao butter and milk fat in proportions indicated by the calculations.

Test fat extracted from sample under examination as directed under (a), but use the prepared mixture of cacao butter and milk fat instead of the pure cacao butter for the blank. If fat being tested contains coconut oil or palm kernel oil, the last filtrate, when acidified, will be more turbid or milky than the blank.

## SILVER NUMBER FOR DETECTION OF COCONUT AND PALM KERNEL OILS (10)—TENTATIVE

## 19.33

## REAGENTS

(a) *Potassium hydroxide soln.*—750 g of KOH/liter.

(b) *Magnesium sulfate soln.*—150 g of  $MgSO_4 \cdot 7H_2O$ /liter.

(c) *Sodium nitrate.*—Crystals as Cl-free as practicable (0.002% or less).

(d) *Ferric indicator.*—Saturated. Use  $FeK(SO_4)_2 \cdot 12H_2O$  or  $FeNH_4(SO_4)_2 \cdot 12H_2O$ .

## 19.34

## DETERMINATION

Weigh 10 g of fat into 250 ml beaker and add 40 ml of alcohol and 5 ml of the KOH soln. Saponify mixture and evaporate to dryness on steam bath. Take up soap in H<sub>2</sub>O (150 ml), warming if necessary. Cool, and make up to 250 ml.

Pipet 200 ml of the soln into 500 ml Erlenmeyer flask. Close flask with stopper carrying thermometer and having small groove lengthwise in side. Place flask in water bath maintained at ca 80°. When sample reaches ca 80°, loosen stopper and introduce 50 ml of the MgSO<sub>4</sub> soln from pipet. Shake flask with rotary motion. Replace stopper and thermometer and allow flask to remain in bath 8–10 min. longer at 70–80°, shaking flask occasionally. Remove flask and cool under tap, with shaking, to 20–25°. Remove stopper and thermometer, stopper tightly, and shake vigorously 4 min. Allow flask to stand in bath at 20–25° until a water layer separates at bottom. Filter thru Büchner funnel, removing all liquid possible by pressing with a horn spoon. Run blank on cacao butter in same manner.

Neutralize 200 ml of filtrate until colorless to phenolphthalein with ca 0.5 *N* H<sub>2</sub>SO<sub>4</sub> soln in 250 ml volumetric flask. Add 20 g of the NaNO<sub>3</sub> crystals and when dissolved add 22.5 ml of 0.2 *N* AgNO<sub>3</sub> soln. Make to mark and shake 3 min. Allow flask to stand short time and filter thru folded filter. To 200 ml of filtrate add 6 ml of the ferric indicator and 4 ml of 40% HNO<sub>3</sub>. Titrate with 0.1 *N* NH<sub>4</sub>SCN to first color change (reddish brown).

Calculate as follows:

Silver number (mg of Ag used/g of fat) =  $(a - b) \times 2.107$ , in which

$a = 1.6 \times \text{ml of } 0.2 \text{ } N \text{ AgNO}_3 \text{ soln added; and}$

$b = \text{ml of } 0.1 \text{ } N \text{ NH}_4\text{SCN soln used in back titration.}$

Factor 2.107:  $\frac{10.788 \text{ (mg Ag/ml } 0.1 \text{ } N \text{ soln)}}{5.12 \text{ (g of fat in aliquot titrated)}}$

The silver number of palm kernel and coconut oils and stearins varies from ca 26 for the stearins to 60 for whole coconut oil. Dairy butter gives a value of ca 11.6, and cacao butter, 0.6.

## CRITICAL TEMPERATURE OF DISSOLUTION OF FAT IN ACETIC ACID (11)—TENTATIVE

## 19.35

## APPARATUS

Insert thermometer reading to 0.1° into cork that fits a  $6 \times \frac{1}{4}$ " test tube and extend it far enough into tube so that bulb will be covered by 10 ml of liquid. Place test tube in larger tube ( $4 \times 1\frac{1}{4}$ ") containing glycerol and hold firmly in place with cork having groove cut in side to equalize pressure when heat is applied.

## 19.36

## DETERMINATION

To remove traces of moisture, filter portion of sample to be examined thru dry paper in oven in which temp. of ca 110° is maintained. Allow filtered sample to cool until barely warm and weigh 5 g of sample and 5 g of 99.5% acetic acid into the test tube. Insert cork holding thermometer and place test tube in glycerol bath. Heat, and shake apparatus frequently until clear soln of the fat and acetic acid is obtained. Allow soln to cool, with constant shaking, without removing from glycerol bath. Note temp. at which first sign of turbidity appears. Make similar test with same acetic acid on sample of pure cacao butter.

As free fatty acids lower turbidity temp., a correction must be made for acid value of sample. If concn of the acetic acid reagent is such that turbidity temp. of the pure cacao butter is ca 90°, one unit of acid value will cause a reduction of 1.4° in the

critical temp. of dissolution. If turbidity temp. is ca 100°, one unit of acid value will cause a reduction of 1.2°. For intermediate temp. reduction is proportional.

Determine acid value (mg of KOH required to neutralize free fatty acids in 1 g of sample) of both sample and pure cacao butter as directed under 31.32, using 5 g of fat. Multiply the acid value by correction factor and add result to observed turbidity temp. Figure obtained is the true critical temp. of dissolution. If this temp. is lower than that of pure cacao butter by more than 3° in the case of fat from chocolate liquors or sweet chocolates, and by more than 6° in the case of fat from milk chocolates, adulteration with coconut, palm kernel, corn, peanut, cottonseed oils, etc., or their stearins, is indicated.

#### 19.37 ACETONE-CARBON TETRACHLORIDE TEST OF FAT (11)—TENTATIVE

Dissolve 5 ml of the warm fat, which has been previously filtered thru dry filter paper in oven at ca 110° to remove traces of moisture, in 5 ml of acetone-CCl<sub>4</sub> (1+1) in test tube. Allow soln to stand in ice water 20–30 min. Run blank on sample of pure cacao butter at same time. If hydrogenated oil, tallow, oleostearin, or paraffin is present, a white flocculent precipitate will soon appear. If the H<sub>2</sub>O is cold enough, cacao butter may solidify. If precipitate is formed, remove sample from ice H<sub>2</sub>O and allow to remain at room temp. for a time. Solidified cacao butter will soon melt and go into soln, but if precipitate is due to any of the above-mentioned possible adulterants a much longer time will be required.

### DAIRY INGREDIENT CONSTITUENTS

#### 19.38 MILK FAT IN MILK CHOCOLATE—TENTATIVE.—See 19.29 and 19.30

#### 19.39 MILK PROTEIN (18)—OFFICIAL

Place 10 g of milk chocolate in centrifuge bottle (250 ml or larger), and extract twice with ca 100 ml of ether by shaking until uniform, centrifuging, and decanting the supernatant ether layer each time. Place in bottle a perforated stopper carrying a bent-glass tube, and a straight glass tube that extends into bottle ca one-third of way to bottom. Expel the ether by applying suction to the bent tube and drawing a moderate current of air thru bottle while it is located in moderately warm (not hot) place. When the ether is expelled, measure 100 ml of H<sub>2</sub>O into bottle with a bulb pipet. Stopper bottle and shake vigorously for 4 min. Measure in with a pipet 100 ml of 1% Na<sub>2</sub>C<sub>2</sub>O<sub>4</sub> soln. Stopper bottle, and shake vigorously 3 min. Allow bottle to stand ca 10 min. and again shake 1–2 min. Place sample in centrifuge and whirl ca 15 min. at high speed (1800 r.p.m. if size 1 type Sb is used).

Remove bottle from centrifuge and decant supernatant liquid into beaker. Pipet 100 ml into dry 250 ml beaker and add 1 ml of acetic acid while stirring gently. Let sample stand for a few min. for the precipitate to partly separate and add 4 ml of 10% tannic acid soln with stirring (soln should not be more than one week old). Allow precipitate to separate and settle a few min., then filter on a Büchner funnel (7 mm size), using moderate suction. The filtrate should be clear. Use as filter a CS & S No. 589 white ribbon paper (or equivalent), overlaid with medium layer of paper pulp, the latter prepared by shaking one 15 ml circle No. 1 Whatman filter paper torn to bits with H<sub>2</sub>O. By means of wash soln (add 1 ml of acetic acid and 2 ml of 10% tannic acid soln to 100 ml of 1% Na<sub>2</sub>C<sub>2</sub>O<sub>4</sub> soln), transfer all precipitate to funnel with aid of policeman. Wash on filter 1 or 2 times. Loosen filter around edge with spatula. Carefully roll up and remove filter and precipitate to Kjeldahl flask. Transfer to flask any particles of precipitate clinging to funnel or spatula with small pieces of damp filter paper. Determine N as directed in 2.26.  $N \times 2 \times 6.38 = \text{total casein and albumin}$

contained in the 10 g taken for analysis. Casein and albumin  $\times 1.07$  = total milk protein.

## 19.40

## LACTOSE (19)—OFFICIAL

(In absence of other reducing sugars)

Determine reducing sugars before inversion as directed under 34.39, in aliquot (usually 20 ml) of the Pb-free filtrate obtained in 19.42. Determine reduced Cu as

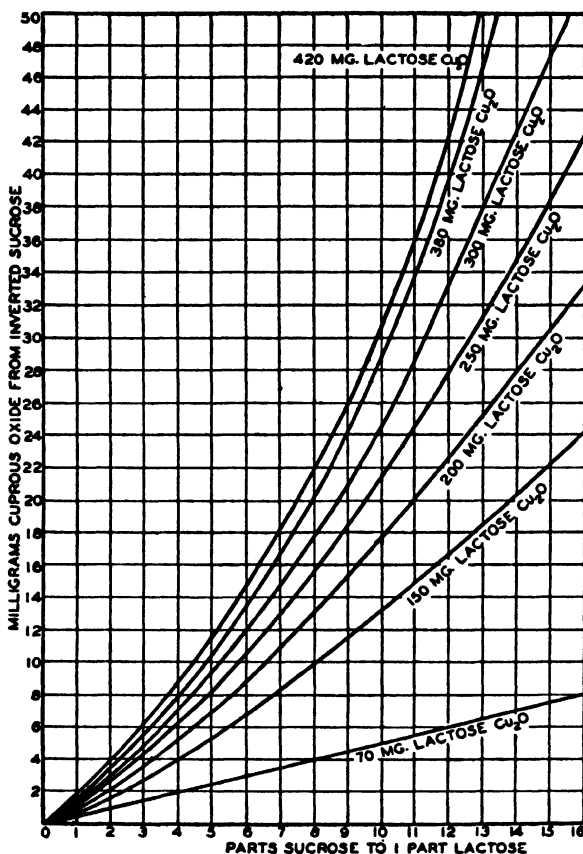


FIG. 26.—GRAPH USED IN CORRECTING CUPROUS OXIDE FOR EFFECT OF SUCROSE

$\text{Cu}_2\text{O}$  by the volumetric thiosulfate method, 34.41. Correct for  $\text{Cu}_2\text{O}$  due to sucrose as follows: Obtain approximate percentage of lactose from following formula, using data obtained in 19.42:

$$\text{Approximate lactose} = \frac{P(1.1 + X/100) - S}{0.79}.$$

From calculated polarimetric sucrose/lactose ratio and total  $\text{Cu}_2\text{O}$  obtained as above, determine amount of  $\text{Cu}_2\text{O}$  to be subtracted from total  $\text{Cu}_2\text{O}$  found, using

graph (Fig. 26). Convert corrected  $\text{Cu}_2\text{O}$  to lactose ( $L$ ), using table, 44.11. Percentage of lactose is then obtained from following relationship:

$$\text{Percentage lactose} = \frac{L(110 + X)}{0.26C}$$

in which  $X$  = value obtained in polarimetric sucrose determination and  $C$  = volume of soln (ml) used in above lactose determination.

## 19.41

## MILK ASH (FROM CALCIUM)—TENTATIVE

Ash 5–10 g of milk chocolate as directed under 34.9 or 34.10. Take up the ash in  $\text{HCl}$  as directed in 33.7, and determine  $\text{CaO}$  as directed under 12.12.  $\text{CaO} \times 5$  = milk ash.

## SACCHARINE INGREDIENTS OTHER THAN LACTOSE

## 19.42

## SUCROSE (14)—OFFICIAL

Transfer 26 g of prepared sample, 19.1, to 8 oz. nursing bottle, add ca 100 ml of petroleum benzene, shake 5 min., and centrifuge. Decant clear solvent carefully and repeat treatment with petroleum benzene. Place bottle containing defatted residue in warm place until petroleum benzene is expelled. Add 100 ml of  $\text{H}_2\text{O}$  and shake until most of chocolate is detached from sides and bottom of bottle. Loosen stopper and carefully immerse bottle 15 min. in water bath kept at  $85\text{--}90^\circ$ , shaking occasionally to remove all chocolate from sides of bottle. Remove from water bath, cool, and add basic  $\text{Pb}$  acetate soln (sp. gr. 1.25) to complete precipitation (5 ml is usually sufficient). Add  $\text{H}_2\text{O}$  to make total volume of 110 ml of added liquid. Mix thoroly, centrifuge, and decant the supernatant liquid thru small filter. Precipitate excess of  $\text{Pb}$  with powdered dry  $\text{K}$  oxalate and filter. Dilute sufficient filtrate with equal volume of  $\text{H}_2\text{O}$ , mix, and polarize in 200 mm tube at  $20^\circ$ , " $P$ ." Obtain invert reading, " $I$ ," at  $20^\circ$  as directed under 34.24(b). Multiply both readings by 2 to correct for dilution. From data obtained calculate percentage of sucrose ( $S$ ) from following formulas:

$$S = \frac{(P - I)(110 + X)}{143.0 - t/2}, \text{ in which}$$

$$X = \frac{0.2244(P - 21d)}{1 - 0.00204(P - 21d)}, \text{ in which}$$

$$d = \frac{P - I}{143.0 - t/2}$$

## 19.43

## DEXTROSE—TENTATIVE

Prepare the clarified and deleded sample soln as directed in 19.42 except to use a sample of only 10 g. Proceed as directed in 34.150 or 34.154.

## 19.44

## CORN SIRUP—TENTATIVE

Prepare the clarified and deleded soln as directed in 19.42 and determine corn sirup as directed in 34.32.

## STARCH

## 19.45

## I. Direct Acid Hydrolysis Method—Tentative

Weigh 4 g of sample if unsweetened, or 10 g if sweetened, into small porcelain

mortar; add 25 ml of ether and grind. After coarser material has settled, decant ether, together with fine suspended matter, on 11 cm paper of sufficiently fine texture to retain crude starch. Repeat this treatment until no more coarse material remains. After ether has evaporated from filter, transfer fat-free residue to mortar by means of jet of cold  $H_2O$  and rub to even paste, filtering on paper previously used. Repeat this process until all sugar is removed. In case of sweetened products filtrate should measure at least 500 ml. Determine crude starch in extracted residue as directed under 27.33.

## 19.46

*II. Diastase Method—Tentative*

Remove fat and sugar from 4 g of sample if unsweetened, or 10 g if sweetened, as directed under 19.45. Carefully wash wet residue into beaker with 100 ml of  $H_2O$ , heat to boiling over asbestos with constant stirring, and continue the boiling and stirring 30 min. Replace  $H_2O$  lost by evaporation and immerse beaker in water bath kept at 55–60°. When liquid has cooled to temp. of bath, add 20 ml of freshly prepared malt extract, 27.34, and digest mixture 2 hours with occasional stirring. Boil a second time for 30 min., dilute, cool, and digest as before with another 20 ml portion of the malt extract. Heat again to boiling, cool, and transfer to 250 ml flask. Add 3 ml of alumina cream, 34.19(b), dilute to mark, and filter thru dry paper. Residue on paper should show no signs of starch when examined microscopically. Continue from this point as directed under 27.35, beginning "Place 200 ml of filtrate in flask, add 20 ml of HCl (sp. gr. 1.125)."

## 19.47

**THEOBROMINE (15)—TENTATIVE**

Extract materials containing considerable fat, such as chocolate liquor or cacao nibs, with petroleum benzine (b. p. below 65°) to remove fat. (This preliminary extraction is unnecessary with samples of cocoas or cacao shell.)

Place 10 g of sample or prepared sample in small porcelain dish. Add 2–3 g of freshly calcined  $MgO$  and mix well with flattened glass rod. Add ca 14 ml (9–20) of  $H_2O$ , a few ml at a time, and triturate carefully and thoroly until every particle is dampened. (Material should be capable of compressing to firm cake.) Place dish containing damp mixture on steam bath for 30 min., mixing at intervals to prevent any part from becoming dry, during which time material should granulate.

At end of 30 min. remove dish and triturate mixture well so that every particle is damp, then transfer to 250 ml flask. Add 150 ml of tetrachlorethane and boil under reflux air condenser for 30 min. Filter nearly boiling hot liquid into second flask of ca 200 ml capacity, preferably having ground-glass joint. (Filtrate should be clear and almost colorless.)

Transfer residue and filter to first flask with 120 ml of the solvent and again reflux 20–30 min. Meanwhile, distil most of liquid in second flask from first extraction thru an air condenser. Filter the hot liquid (2nd extraction) into second flask and repeat process of refluxing and distillation twice more, using 120 ml portions of the tetrachlorethane. Receive filtrates from all extractions in flask two, intermittently distilling off portions as directed above. Distil liquid after last extraction until reduced to volume of 3–5 ml.

Cool flask and residue and add with rotation 65 ml of ether, mix well, stopper, and allow to stand at least 1 hour (until supernatant liquid is clear). Collect precipitate on tared filter paper, using several 5–7 ml portions of ether to transfer and wash. Dry filter and precipitate at 100° and weigh. Add to weight found .004 g to compensate for theobromine dissolved in the ether. Calculate percentage of theobromine in original material.



## SELECTED REFERENCES

- (1) Federal Register, October 19, 1940, p. 4152.
- (2) J. Assoc. Official Agr. Chem., 14, 530 (1931); 16, 66 (1933).
- (3) Ibid., 28, 76, (1945).
- (4) Ibid., 24, 74 (1941).
- (5) Ibid., 9, 46 (1926).
- (6) Ibid., 10, 42 (1927).
- (7) Ibid., 13, 43 (1930).
- (8) Ibid., 11, 45 (1928).
- (9) Ibid., 13, 45, 78, 486 (1930).
- (10) Ibid., 15, 549 (1932); 17, 64, 375 (1934).
- (11) Ibid., 5, 263 (1921); 7, 150 (1923).
- (12) Ibid., 22, 603 (1939); 24, 715 (1941); 25, 716 (1942).
- (13) Ibid., 16, 566 (1933); 17, 65, 377 (1934).
- (14) Ibid., 16, 565 (1933); 17, 65 (1934).
- (15) Analyst, 46, 35 (1921).

## 20. CEREAL FOODS

### WHEAT FLOUR (1)

#### 20.1

##### DIRECTIONS FOR SAMPLING—OFFICIAL

Sample a number of sacks equivalent to square root of number in lot, but not less than 10, *i.e.*, 10 from 100 or less, 15 from 225, 20 from 400 sacks, etc.

Select sacks to be sampled according to their exposure in ratio of 4 from most exposed, 3 from next less exposed, 2 from next, and 1 from the least exposed portion of the lot.

From each sack to be sampled, draw a core from one corner of top diagonally to center of sack by means of cylindrical, pointed, polished metal trier,  $\frac{1}{2}$ " in diam., with slit at least  $\frac{1}{2}$  the circumference. Draw a second core from other top corner to  $\frac{1}{2}$  distance to center of sack.

Deliver the 2 cores at once to a clean, dry, air-tight container, which has stood open for a few minutes near the lot of flour to be sampled, and seal immediately. Use separate container for each sack sampled. One of following containers may be used: (1) Pint fruit jar provided with rubber gasket; (2) rubber pouch which can be tied or sealed to exclude moisture or air; (3) tin can or box with moisture and air-tight friction top.

Before opening sample for analysis, alternately invert and roll each container 25 times, or more if necessary, to secure homogeneous mixture. Avoid extreme temps. and humidities when opening containers for analysis. Keep sample tightly sealed at all other times.

##### TOTAL SOLIDS (MOISTURE, INDIRECT METHOD)

##### *Vacuum Oven Method (2)—Official*

#### 20.2

##### APPARATUS

(a) *Metal dish*.—Diameter ca 55 mm, height ca 15 mm, provided with inverted slip-in cover fitting tightly on inside.

(b) *Air-tight desiccator*.—Reignited CaO is a satisfactory drying agent.

(c) *Vacuum oven*.—Connect with pump capable of maintaining partial vacuum in oven with pressure equivalent to 25 mm or less of Hg and provided with thermometer passing into oven in such a way that bulb is near samples. Connect H<sub>2</sub>SO<sub>4</sub> gas-drying bottle with oven for admitting dry air when releasing vacuum.

#### 20.3

##### DETERMINATION

Weigh accurately ca 2 g of the well-mixed sample in covered dish that previously has been dried at 98–100°, cooled in desiccator, and weighed soon after attaining room temp. Loosen the cover (do not remove) and heat at 98–100° to constant weight (ca 5 hours) in partial vacuum having pressure equivalent to 25 mm or less of Hg. Admit dry air into oven to bring to atmospheric pressure. Immediately tighten cover on dish, transfer to desiccator, and weigh soon after room temp. is attained. Report flour residue as total solids and loss in weight as moisture (indirect method).

#### 20.4

##### *Air-Oven Method (3)—Official*

(Results closely approximate those obtained by vacuum method)

In a cooled and weighed dish (provided with cover) that has been previously

heated to  $130^{\circ} (\pm 3^{\circ})$ , weigh accurately ca 2 g of the well-mixed sample. Uncover sample, and dry dish, cover, and contents an hour in an oven provided with opening for ventilation and maintained at  $130^{\circ} (\pm 3^{\circ})$ . (1 hour drying period begins when oven temp. is actually  $130^{\circ}$ .) Cover dish while still in oven, transfer to desiccator, and weigh soon after room temp. is attained. Report flour residue as total solids and loss in weight as moisture (indirect method).

#### ASH (4)

##### 20.5

#### *Method I—Official*

Weigh 3–5 g of the well-mixed sample into shallow, relatively broad ashing dish that has been ignited, cooled in desiccator, and weighed soon after attaining room temp. Incinerate in furnace at ca  $550^{\circ}$  (dull red) until light gray ash results, or to constant weight. Cool in desiccator and weigh soon after room temp. is attained. Reignited CaO is a satisfactory drying agent for desiccator.

#### *Method II—Magnesium Acetate Method (5)—Official*

##### 20.6

#### REAGENT

*Magnesium acetate soln.*—Dissolve 4.054 g of  $\text{Mg}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 4\text{H}_2\text{O}$  in 50 ml of  $\text{H}_2\text{O}$  and make up to 1 liter with alcohol.

##### 20.7

#### DETERMINATION

From a buret add 5 ml of the reagent to 3–5 g of flour, bread, etc., or 10 ml to 1 g sample of bran, wheat germ, etc. Allow mixture to stand 1–2 min., evaporate excess alcohol, and place sample in muffle furnace maintained at  $700^{\circ}$ , closing door after flaming has ceased. When incineration is complete, place ashing dish in desiccator until cool, then weigh. Run blank determination on soln and deduct blank from weight of crude ash. Evaporate blank cautiously.

#### ORIGINAL ASH OF FLOUR IN PHOSPHATED AND SELF-RISING FLOUR (6)

##### 20.8

#### *Gustafson Method—Tentative*

To 20–25 g of the flour in metal centrifuge tube (cup 2" in diam., 6" deep), add sufficient  $\text{CCl}_4$  to fill tube to within 1" of top (ca 250 ml). Centrifuge 5–7 min. at 1,600 r.p.m., and allow centrifuge to come to rest slowly. Carefully skim off flour, which is now in compact layer on surface of the  $\text{CCl}_4$ , with large tablespoon, recovering as much of the flour as is possible in one spoonful. (With care, ca 90% of original flour may be recovered.) Allow wet flour to dry overnight and proceed as directed under 20.5. (The  $\text{CCl}_4$  may be filtered, distilled, and used again.)

#### IRON (7)—OFFICIAL, FIRST ACTION

(Applicable to enriched, enriched self-rising, and phosphated flours)

##### 20.9

#### PREPARATION OF SAMPLE

Slice bread, allow to air-dry until in equilibrium with air, and crush to ca 20-mesh size on wooden surface with wooden rolling pin. (Grinding may be done in mill if experiments show no increase in Fe due to grinding on the particular material under examination. In general, grinding in mills increases the iron content.)

##### 20.10

#### REAGENTS

(a) *Orthophenanthroline soln.*—Dissolve 0.1 g of orthophenanthroline in ca 80 ml of  $\text{H}_2\text{O}$  at  $80^{\circ}$ , and after cooling dilute to 100 ml.

(b) *Alpha-alpha dipyridyl soln.*—Dissolve 0.1 g of alpha-alpha dipyridyl (Eastman Co.) in  $\text{H}_2\text{O}$  and dilute to 100 ml.

(Keep Reagents (a) and (b) in cool, dark place and they will remain stable for several weeks.)

(c) *Hydroxylamine hydrochloride soln.*—Dissolve 10 g of hydroxylamine hydrochloride in  $\text{H}_2\text{O}$  and dilute to 100 ml.

(d) *Magnesium nitrate soln.*—Dissolve 50 g of  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and dilute to 100 ml.

(e) *Acetate buffer soln.*—Dissolve 8.3 g of C.P. anhydrous Na acetate (previously dried at  $100^\circ$ ) in  $\text{H}_2\text{O}$ , add 12 ml of acetic acid, and dilute to 100 ml. (It may be necessary to redistil the acetic acid and purify the Na acetate by recrystallization from  $\text{H}_2\text{O}$ , depending on amount of Fe present.)

## 20.11

### PREPARATION OF REFERENCE CURVE

(1) Dissolve 0.1 g of analytical grade Fe wire in 20 ml of HCl and 50 ml of  $\text{H}_2\text{O}$ , and dilute to 1 liter. Dilute 100 ml of this soln to 1 liter. Each ml contains 0.01 mg of Fe. Or—

(2) Dissolve 3.512 g of  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ , add 2 drops of HCl, and dilute to 500 ml. Dilute 10 ml of this soln to 1 liter. Each ml contains 0.01 mg of Fe.

Prepare 10 solns containing 2.0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, and 45.0 ml, respectively, of the finally diluted stock soln, and 2.0 ml of HCl diluted to 100 ml. Also prepare a blank soln containing only the HCl. Use 10 ml of each of these solns and follow the procedure as given under 20.12, beginning "add 1 ml of the hydroxylamine soln." Plot concentration against scale reading.

## 20.12

### DETERMINATION

Ash 10.0 g of flour or air-dry bread in Pt,  $\text{SiO}_2$ , or porcelain dish (ca 60 mm diam., 35 ml capacity) in accordance with method for ash, 20.5. (Porcelain evaporating dishes of ca 25 ml capacity are satisfactory. Do not use flat-bottomed dishes of greater diameter than 60 mm.) Cool, and weigh if percent ash is desired. Continue ashing until practically C-free. To diminish ashing time, or for samples that do not burn practically C-free, use one of the following ash aids: Moisten the ash with 0.5–1.0 ml of the  $\text{Mg}(\text{NO}_3)_2$  soln or with distilled  $\text{HNO}_3$ . Dry contents and carefully ignite in muffle to prevent spattering. (A white ash with no C results in most cases.) Do not add these ash aids to self-rising flour or bread (products containing NaCl) in a Pt dish because of vigorous action on the dish. Cool, add 5 ml of HCl, allowing the acid to rinse the upper portion of the dish, evaporate to dryness on steam bath, dissolve residue by adding, accurately measured, 2.0 ml of HCl, heat for 5 min. on steam bath with watch-glass on dish, wash off watch-glass with  $\text{H}_2\text{O}$ , filter into 100 ml volumetric flask, cool, and dilute to volume. Pipet 10 ml aliquot into 25 ml volumetric flask, and add 1 ml of the hydroxylamine soln; in a few minutes add 5 ml of the buffer soln and 1 ml of the ortho-phenanthroline or 2 ml of the alpha-alpha dipyridyl soln and make to volume. Read intensity of color in 2" cell in neutral wedge photometer, using the No. 51 filter (ca 510 wave length). (Another suitable instrument of equivalent precision may be used.) From the reading, determine concentration of Fe from equation of line representing the standard points or by reference to standard curve for known Fe concentration. Determine blank on reagents and make correction. Calculate amount of Fe in flour or bread as mg per pound. Rinse all flasks, beakers, funnels, etc., with  $\text{H}_2\text{O}$  before use, and filter all reagents to remove suspended matter.

## 20.13

## CALCIUM (8)—OFFICIAL, FIRST ACTION

(Applicable to enriched, enriched self-rising, and phosphated flours)

Ash 10 g of flour or air-dried bread as directed in 20.5, and proceed as directed in 20.12 as far as "filter into 100 ml volumetric flask"; instead filter into 400 ml beaker or transfer 50 ml of the soln from the Fe determination to 400 ml beaker and dilute to ca 150 ml.

Add 8–10 drops of bromocresol green indicator and sufficient 20% Na acetate soln to change pH to 4.8–5.0 (blue). Cover with watch-glass and heat to boiling. Precipitate the Ca slowly by adding 3% oxalic acid soln, a drop every 3–5 seconds, until pH is changed back to 4.4–4.6 (optimum for Ca oxalate precipitation) as indicated by appearance of distinct green shade. (Change of color will indicate excess of oxalic acid—more would develop yellow tints, showing an undesirable displacement of the pH.) Boil 1–2 min. and allow mixture to settle until clear, or overnight. Filter supernatant liquid thru quantitative filter paper or Gooch crucible or on fritted glass filter (Jena 1G4 is preferable) and wash beaker and precipitate with ca 50 ml of  $\text{NH}_4\text{OH}$  (1+50) in small portions, using a wash bottle delivering very small stream. Break point of filter and wash filter or crucible with mixture of 125 ml of  $\text{H}_2\text{O}$  and 5 ml of  $\text{H}_2\text{SO}_4$  at 80–90°. Titrate at temp. of 70–90° with 0.05 N  $\text{KMnO}_4$  until slight pink color is obtained, add filter paper, and continue titration if necessary. Correct for blank and calculate Ca as mg/lb. 1 ml of 0.05 N  $\text{KMnO}_4$  = 1 mg of Ca.

## 20.14

## TOTAL CARBON DIOXIDE IN SELF-RISING FLOUR (9)—OFFICIAL

(Not applicable with added calcium carbonate)

Use 17 g of flour, 15–20 glass beads (4–6 mm diam.), and 45 ml of  $\text{H}_2\text{SO}_4$  (1+5), and proceed as directed under 17.4–17.6, as far as the calculation, except to agitate the flask vigorously 3 min. and allow it to stand 10 min. to secure equilibrium. Calculate as follows: Subtract volume of acid used from total buret reading and correct for temp. and pressure. Divide corrected reading by 100 to obtain percentage of  $\text{CO}_2$  (by weight). Correct apparent per cent of  $\text{CO}_2$  to compensate for varying atmospheric conditions by immediately assaying a synthetic sample of known composition and like ingredients by the same method in the same apparatus. Divide weight of  $\text{CO}_2$  recovered from synthetic sample by weight of  $\text{CO}_2$  contained in  $\text{NaHCO}_3$  used and record quotient. Apparent per cent of total  $\text{CO}_2$  in official sample ÷ this quotient = corrected per cent total  $\text{CO}_2$  in official sample.

## 20.15

## CRUDE FAT OR ETHER EXTRACT—OFFICIAL

Proceed as directed under 27.25. With fine flour the addition of an equal weight of clean, dry sand may be necessary.

## 20.16

## FAT (ACID HYDROLYSIS METHOD) (10)—OFFICIAL

Place 2 g of the flour in 50 ml beaker, add 2 ml of alcohol, and stir so as to moisten all particles. (Moistening of sample with alcohol prevents lumping on addition of the acid.) Add 10 ml of  $\text{HCl}$  (25+11), mix well, set beaker in water bath held at 70–80°, and stir at frequent intervals 30–40 min. Add 10 ml of alcohol and cool. Transfer mixture to Röhrig or Mojonnier fat extraction apparatus. Rinse beaker into extraction tube with 25 ml of ether in 3 portions, stopper flask with cork, Neoprene, or other synthetic rubber stopper not affected by solvents, and shake vigorously 1 min. Add 25 ml of redistilled petroleum benzine (b.p. below 60°) and again shake vigorously 1 min. Let stand until upper liquid is practically clear or centrifuge Mojonnier flask 20 min. at ca 600 r.p.m. Draw off as much as possible of ether-fat

soln thru filter consisting of pledget of cotton packed just firmly enough in stem of funnel to allow free passage of ether into weighed 125 ml beaker-flask containing some porcelain chips or broken glass. Before weighing beaker-flask dry it and a similar flask as a counterpoise in drying oven at 100° and then allow it to stand in air to constant weight. Re-extract liquid remaining in tube twice, each time with only 15 ml of each ether. Shake well on addition of each ether. Draw off clear ether solns thru filter into same flask as before and wash tip of spigot, funnel, and end of funnel stem with a few ml of a mixture of the 2 ethers in equal volumes free from suspended H<sub>2</sub>O. Evaporate ethers slowly on steam bath, then dry fat in drying oven at 100° to constant weight (ca 90 min.). Remove flask and counterpoise from oven, allow to stand in air to constant weight (ca 30 min.), and weigh. (Owing to size of flask and nature of the material, there is less error by cooling in air than in desiccator.) Correct this weight by blank determination on reagents used. Report as % fat by acid hydrolysis.

## 20.17

CRUDE FIBER—OFFICIAL.—See 27.28–27.30

## FAT ACIDITY (11)—TENTATIVE

## 20.18

## REAGENTS

(a) *Benzene-alcohol-phenolphthalein soln.*—To 1 liter of C<sub>6</sub>H<sub>6</sub> add 1 liter of alcohol and 0.4 g of phenolphthalein to form 0.02% soln.

(b) *Alcohol-phenolphthalein soln.*—To 1 liter of alcohol add 0.4 g of phenolphthalein (0.04% soln).

(c) *Potassium hydroxide soln.*—0.0178 N. Carbonate free. 1 ml of this soln contains 1 mg of KOH.

## 20.19

## APPARATUS

(a) *Grain mill.*—Suitable mill for grinding small samples.

(b) *Sieve.*—40-mesh.

(c) *Drying oven.*—Capable of maintaining temp. of boiling H<sub>2</sub>O.

(d) *Fat extraction apparatus.*—Soxhlet or other suitable type. (Double paper thimbles or alundum R. A. 360 thimbles are suitable for extraction purposes.)

## DETERMINATION

## 20.20

## I. Applicable to All Cereal Grains and Flour

Obtain a representative sample of ca 50 g of the grain (corn 100 g) by hand quartering or by use of mechanical sampling device. Preferably so grind sample that at least 90% will pass thru 40-mesh sieve (somewhat coarser grind will not materially affect results). If sample is too moist to grind readily, dry at a temp. of ca 100° only long enough to remove excess moisture.

Extract 10 g ( $\pm 0.01$  g) of ground sample with petroleum benzene ca 16 hours in the extractor. Start extraction as soon as possible after sample has been ground, and under no circumstances allow ground sample to remain overnight. Completely evaporate solvent from extract on steam bath. Dissolve residue in extraction flask with 50 ml of the benzene-alcohol-phenolphthalein soln. Titrate dissolved extract with the KOH soln to a distinct pink color, or in case of a yellow soln to an orange-pink. If emulsion forms during titration, dispel by addition of a second 50 ml portion of the benzene-alcohol-phenolphthalein soln. End point should match color of a soln made by adding 2.5 ml of 0.01% KMnO<sub>4</sub> soln to 50 ml of a K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> soln of proper strength to match the color of original soln being titrated. (Add a 0.5% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> soln dropwise to 50 ml of H<sub>2</sub>O until color is matched. Then add 2.5 ml of the 0.01% KMnO<sub>4</sub>

soln. This final soln may be used as the standard for titration end point of extract.) Make blank titration on 50 ml of the benzene-alcohol-phenolphthalein soln and subtract this value from titration value of sample. If the additional 50 ml portion of the benzene-alcohol-phenolphthalein soln has been added, double the blank titration. Report fat acidity as mg of the KOH required to neutralize free fatty acids from 100 g of grain (dry basis). Fat acidity =  $10 \times (\text{titration} - \text{blank})$ .

## 20.21

*II. Rapid Method for Corn*

(Results may be obtained in less than 1 hour.)

Prepare sample as directed in 20.20. Weigh 20 g ( $\pm 0.01$  g) into 100 ml glass-stoppered flask or bottle. Add exactly 50 ml of  $C_6H_6$ , insert stopper, shake a few seconds to saturate air in flask with benzene vapor, momentarily loosen stopper to release pressure, and replace stopper. Shake flask 30 min. in a mechanical shaker, or periodically by hand for 45 min. Tilt flask and allow meal to settle at an angle for at least 3 min. Carefully decant as much of liquid as possible into 15 cm folded filter paper inserted in 8 cm glass funnel, and cover funnel with cover-glass to minimize evaporation. Collect exactly 25 ml of filtrate in 25 ml volumetric flask. Then transfer this filtrate to 250 ml Florence flask. Refill the volumetric flask to 25 ml mark with the alcohol-phenolphthalein soln and transfer to flask containing benzene extract.

Using color standard prepared as in 20.20, titrate extract with the standard KOH soln to distinct pink color in case of white corn, and to orange-pink for yellow corn. If emulsion forms during titration, dispel by addition of 25 ml each of benzene and of the alcohol-phenolphthalein soln. Run blank titration on mixture of 25 ml of  $C_6H_6$  and 25 ml of the alcohol-phenolphthalein soln. If the additional benzene and alcohol have been added, double the blank titration. Report fat acidity as mg of KOH required to neutralize free fatty acids from 100 g of corn (dry basis). Fat acidity =  $10 \times (\text{titration} - \text{blank})$ , calculated on dry basis.

## H-ION CONCENTRATION

*Colorimetric Method—Official*

## 20.22

## PREPARATION OF SULFONPHTHALEIN INDICATORS (12)

	A	pH
Bromocresol green	14.3	3.8–5.4
Chlorophenol red	23.6	4.8–6.4
Bromothymol blue	16.0	6.0–7.6
Phenol red	28.2	6.8–8.4

A = ml of 0.01 N NaOH/0.1 g of indicator to form monosodium salt. Dilute to 250 ml for 0.04% reagent.

## 20.23

## PREPARATION OF STOCK SOLUTIONS

Use recently boiled  $H_2O$ .

(a) *Acid potassium phthalate soln.*—0.2 M. Dry the C. P. salt to constant weight at 110–115°. Dissolve 40.836 g in  $H_2O$  and dilute to 1 liter.

(b) *Monopotassium phosphate soln.*—0.2 M. Dry  $KH_2PO_4$  to constant weight at 110–115°. Dissolve 27.232 g in  $H_2O$  and dilute to 1 liter. The soln should be distinctly red with methyl red, and distinctly blue with bromophenol blue.

(c) *Boric acid-potassium chloride soln.*—0.2 M. Dry  $H_3BO_3$  to constant weight in desiccator over  $CaCl_2$ . Dry KCl in oven at 115–120° for 2 days. Dissolve 12.405 g of  $H_3BO_3$  and 14.912 g of KCl in  $H_2O$  and dilute to 1 liter.

(d) *Sodium hydroxide soln.*—0.2 M. Should be as free as possible from carbonate.

Dissolve 100 g of NaOH in 100 ml of  $H_2O$ , and allow to stand overnight till carbonate has settled. Pipet clear soln from sediment and dilute to a soln somewhat more concentrated than normal. Standardize this soln with an acid soln of known concn, or with a sample of  $KHC_8H_4O_4$ . From this approximate standardization calculate the quantity required for a 0.2 *M* soln. Make required dilution with least possible exposure and pour soln into Pyrex glass bottle. Carefully standardize the soln. The simplest method of doing this is by means of the  $KHC_8H_4O_4$ . 0.04084 g of  $KHC_8H_4O_4$  = 1 ml of 0.2 *M* NaOH. It is preferable to use a factor with the soln rather than attempt adjustment to an exact 0.2 *M* soln. Use phenolphthalein as indicator.

## 20.24

## PREPARATION OF BUFFER SOLUTIONS

The maximum range of standard buffer solns usually needed in cereal work is pH 5.0–8.6. Prepare these from the following stock solns, and in each case dilute to 200 ml.

*Phthalate-NaOH Mixtures*

pH	0.2 <i>M</i> KH PHTHALATE (ML)	0.2 <i>M</i> NaOH (ML)
5.0	50	23.65
5.2	50	29.75
5.4	50	35.25
5.6	50	39.70
5.8	50	43.10
6.0	50	45.40
6.2	50	47.00

*KH<sub>2</sub>PO<sub>4</sub>-NaOH Mixtures*

pH	0.2 <i>M</i> KH <sub>2</sub> PO <sub>4</sub> (ML)	0.2 <i>M</i> NaOH (ML)
5.8	50	3.66
6.0	50	5.64
6.2	50	8.55
6.4	50	12.60
6.6	50	17.74
6.8	50	23.60
7.0	50	29.54
7.2	50	34.90
7.4	50	39.34
7.6	50	42.74
7.8	50	45.17
8.0	50	46.85

*H<sub>3</sub>BO<sub>3</sub>-KCl-NaOH Mixtures*

pH	0.2 <i>M</i> H <sub>3</sub> BO <sub>3</sub> , KCl (ML)	0.2 <i>M</i> NaOH (ML)
7.8	50	2.65
8.0	50	4.00
8.2	50	5.90
8.4	50	8.55
8.6	50	12.00

## 20.25

## PREPARATION OF COLORIMETRIC STANDARDS

Place 20 ml of the buffered soln in ampuls  $\frac{1}{4}$ " in diam., or in test tubes of similar bore, and add 0.5 ml of indicator soln. Do not keep the ampuls or tubes longer than a few days unless sealed, because the buffer solns may spoil.



## 20.26

## DETERMINATION

To 10 g of the sample add 100 ml of cool, recently boiled distilled  $H_2O$  and digest at  $25^\circ$  for 30 min., shaking occasionally during digestion period. Allow mixture to stand quietly for 15 min. and then decant supernatant liquid thru a folded, hardened, dry filter paper. Discard first 5 ml to come thru, but catch the next 60 ml, 20 ml in each of three tubes exactly like the tubes holding the colorimetric standards. Add 0.5 ml of the proper indicator to one tube and compare the resultant color with the prepared standards to determine the pH.

A somewhat crude but helpful application of Walpole's principle to compensate for color and turbidity of sample can be made from a block of wood. Bore parallel and in pairs, 6 deep holes, each large enough to hold one color standard or sample tube. Place adjacent pairs as close together as possible without breaking thru intervening walls. Perpendicular to these holes and running thru each pair bore smaller holes, thru which the test tubes may be viewed. The center pair of test tubes holds soln to be tested plus indicator and also a water blank. At each side place the standards colored with the indicator, and back each by a sample of soln being tested. Place the light on the side of the comparator containing the two controls and water blank. If daylight is used, the light from the northern sky is best. If artificial light is used it must not be too brilliant and should be passed thru daylight type of glass. For average conditions a light intensity of 15–20 microamperes as registered thru a photronic cell is adequate.

## 20.27

*Electrometric Method (13)—Official, First Action*

Weigh 10.0 g of sample into clean, dry Erlenmeyer flask and add 100 ml of recently boiled, distilled  $H_2O$  at  $25^\circ$ . Shake contents of flask until particles are evenly suspended and mixture is free of lumps. Digest for 30 min., shaking the suspension at frequent intervals. Let stand for 10 additional min., decant the supernatant liquid into the H-ion vessel, and immediately determine pH, using an electrode and potentiometer that have been standardized by buffer solns of pH 4.01 (0.05 *M* soln of acid potassium phthalate (NBS Standard Sample 84b) and of pH 9.18 (0.01 *M*  $Na_2B_4O_7 \cdot 10H_2O$ ), both at  $25^\circ$ . (The  $Na_2B_4O_7$  should be recrystallized at least once (14)).

✓ **REDUCING AND NON-REDUCING SUGARS IN FLOUR (15)—OFFICIAL**

## 20.28

## REAGENTS

(a) *Acid buffer soln.*—Make 3 ml of acetic acid, 4.1 g of anhydrous Na acetate, and 4.5 ml of  $H_2SO_4$  to 1 liter with  $H_2O$ .

(b) *Sodium tungstate soln.*—12%. Make 12.0 g of  $Na_2WO_4 \cdot 2H_2O$  to 100 ml with  $H_2O$ .

(c) *Ferricyanide soln (alkaline).*—0.1 *N*. 33.0 g of pure dry  $K_3Fe(CN)_6$  and 44.0 g of anhydrous  $Na_2CO_3$  per liter.

(d) *Acetic acid-salt mixture.*—Make up 200 ml of acetic acid, 70 g of KCl, and 40 g of  $ZnSO_4 \cdot 7H_2O$  to 1 liter with  $H_2O$ .

(e) *Soluble starch-potassium iodide soln.*—Add 2 g of soluble starch to small quantity of cold  $H_2O$  and pour slowly into boiling  $H_2O$  with constant stirring. Cool thoroly (or resulting mixture will be dark colored), add 50 g of KI and make up to 100 ml with  $H_2O$ . Add 1 drop of NaOH soln (1 + 1). Use 1 ml.

(f) *Thiosulfate soln.*—0.1 *N*. 24.82 g of  $Na_2S_2O_5 \cdot 5H_2O$  and 3.8 g of  $Na_2B_4O_7 \cdot 10H_2O$  per liter.

Make "blank" determination with each day's run of sugar determinations to guard against changes in the  $K_3Fe(CN)_6$  and to make correction for any reducing

impurities in the reagents as follows: Combine 5 ml of alcohol, 50.0 ml of the acid buffer soln, and 2 ml of the Na tungstate soln. To 5 ml of this mixture (used in place of 5 ml of flour extract) add 10.0 ml of the  $K_3Fe(CN)_6$  soln and proceed as directed for reducing sugars. (10.0 ml of the  $Na_2S_2O_3$  soln should discharge the blue starch-I color.) If the titration falls within  $30 \pm .05$  ml do not discard the reagents but make correction in subsequent sugar calculations by using the  $Na_2S_2O_3$  equivalent of 10 ml of  $K_3Fe(CN)_6$  soln (i.e., ml of  $Na_2S_2O_3$  soln required in above titration) instead of 10.0 as basis for subtraction.

## 20.30

*0.1 N Ferricyanide Maltose-sucrose Conversion Table\**

0.1 N FERRICYANIDE REDUCED	MALTOSE PER 10 g OF FLOUR	SUCROSE PER 10 g OF FLOUR	0.1 N FERRICYANIDE REDUCED	MALTOSE PER 10 g OF FLOUR	SUCROSE PER 10 g OF FLOUR
ml	mg	mg	ml	mg	mg
0.10	5	5	4.50	237	214
0.20	10	10	4.60	244	218
0.30	15	15	4.70	251	223
0.40	20	19	4.80	257	228
0.50	25	24	4.90	264	233
0.60	31	29	5.00	270	238
0.70	36	34	5.10	276	242
0.80	41	38	5.20	282	247
0.90	46	43	5.30	288	251
1.00	51	48	5.40	295	256
1.10	56	52	5.50	302	261
1.20	60	57	5.60	308	266
1.30	65	62	5.70	315	270
1.40	71	67	5.80	322	275
1.50	76	71	5.90	328	280
1.60	80	76	6.00	334	285
1.70	85	81	6.10	341	290
1.80	90	86	6.20	347	294
1.90	96	91	6.30	353	299
2.00	101	95	6.40	360	304
2.10	106	100	6.50	367	309
2.20	111	104	6.60	373	313
2.30	116	109	6.70	379	318
2.40	121	114	6.80	385	323
2.50	126	119	6.90	392	328
2.60	130	123	7.00	398	333
2.70	135	128	7.10	406	337
2.80	140	133	7.20	412	342
2.90	145	138	7.30	418	347
3.00	151	143	7.40	425	352
3.10	156	148	7.50	431	357
3.20	161	152	7.60	438	362
3.30	166	157	7.70	445	367
3.40	171	161	7.80	451	372
3.50	176	166	7.90	458	377
3.60	182	171	8.00	465	382
3.70	188	176	8.10	472	387
3.80	195	181	8.20	478	392
3.90	201	185	8.30	485	397
4.00	207	190	8.40	492	402
4.10	213	195	8.50	499	407
4.20	218	200	8.60	505	—
4.30	225	204	8.70	512	—
4.40	231	209	8.80	519	—

\* These values are arbitrarily given for 10 g of flour altho the determination is made on only 0.5 g of flour.

## 20.29

## DETERMINATION

(a) *Preparation of extract*.—Introduce 5.675 g of flour into 100 or 125 ml Erlenmeyer flask. Tip flask so that all the flour is at one side, then wet flour with 5 ml of alcohol. Tip flask so that the wet flour is at the upper side and add 50.0 ml of the acid buffer soln, keeping soln from coming in contact with the flour until all has been added to the flask. Then shake flask to bring flour into suspension. Add immediately 2 ml of the Na tungstate soln and again mix thoroly. Filter at once (Whatman No. 4 or equivalent), discarding first 8–10 drops of filtrate.

(b) *Reducing sugars*.—Pipet 5 ml of the flour extract into a test tube of ca 75 ml capacity (Pyrex 1×8"). Add to test tube exactly 10 ml of the  $K_3Fe(CN)_6$  soln, mix, and immerse test tube in a vigorously boiling water bath (surface of liquid in test tube should be 3–4 cm below surface of boiling  $H_2O$ ).

After test tube has been in boiling water bath exactly 20 min., cool tube and contents under running  $H_2O$ , and pour at once into a 100 or 125 ml Erlenmeyer flask. Rinse out test tube with 25 ml of the acetic acid-salt soln, add to contents of Erlenmeyer flask, and mix thoroly. Then add 1 ml of the starch-KI soln. Titrate with the  $Na_2S_2O_3$  soln to complete disappearance of blue color (10 ml micro-buret recommended). Subtract the ml of 0.1 N  $Na_2S_2O_3$  used in titration from 10.00. In case there is a slight blank in the  $K_3Fe(CN)_6$ - $Na_2S_2O_3$  titration make correction by subtracting from the  $Na_2S_2O_3$  equivalent of  $K_3Fe(CN)_6$  soln, 20.28(f). This difference represents a definite quantity of reducing sugar per 10 g of flour which may be ascertained (as maltose) by consulting the table, 20.30.

(c) *Non-reducing sugars*.—Pipet 5 ml of the flour extract into an 8" test tube and immerse in vigorously boiling water bath. After boiling 15 min. cool test tube and contents under running  $H_2O$  and add exactly 10 ml of the  $K_3Fe(CN)_6$  soln. Proceed as directed under (b).  $K_3Fe(CN)_6$  reduced after hydrolysis— $K_3Fe(CN)_6$  reduced by the maltose in the flour = non-reducing sugars calculated as sucrose and obtained from the table, 20.30.

## 20.31

## PROTEIN—OFFICIAL

Determine N as directed under 2.24, 2.25, or 2.26, and multiply percentage of N by 5.7 to obtain percentage of protein. Use factor 5.7 to convert N to protein in wheat used either for manufacturing purposes or for human food.

## 20.32

## 70 PER CENT ALCOHOL-SOLUBLE PROTEINS—TENTATIVE

Transfer 4 g of flour to 150–200 ml bottle or Erlenmeyer flask and add 100 ml of alcohol, 70% by volume, taking care that none of material adheres to bottom of container. Shake thoroly 10–12 times at intervals of 30 min. at room temp., or shake continuously in shaking machine 1 hour, and set aside overnight. Again shake thoroly and filter thru dry, folded filter, returning first runnings to filter until clear filtrate is obtained. Pipet 50 ml of filtrate, equivalent to 2 g of sample, into Kjeldahl flask; dilute with 100 ml of  $H_2O$  to prevent frothing during digestion; determine N as directed under 2.24, 2.25, or 2.26, and convert to protein by factor 5.7. Make blank determination on reagents.

## 20.33 PROTEIN SOLUBLE IN 5 PER CENT POTASSIUM SULFATE SOLUTION—TENTATIVE

Weigh 6 g of flour into 200 ml flask and introduce exactly 100 ml of 5%  $K_2SO_4$  soln. Shake at 30 min. intervals 3 hours or, better, agitate at moderate speed in mechanical shaker 1 hour; let settle 30 min., and filter. Determine N in 50 ml of filtrate as directed under 2.25 or 2.26, making allowance for N contained in reagents, and convert to protein by factor 5.7.

**20.34 PROTEIN SOLUBLE IN SODIUM CHLORIDE SOLUTION AND NON-PROTEIN NITROGEN (16)—TENTATIVE**

Weigh 10 g of flour into 500 ml Erlenmeyer flask, add 250 ml of 1% NaCl soln, stopper flask, and shake thoroly. Let stand, with occasional shaking, 3 hours; filter and evaporate 100 ml of filtrate to small volume in Kjeldahl digestion flask with 5 ml of  $\text{H}_2\text{SO}_4$ . Add 25 ml more acid and determine N as directed under 2.24, 2.25, or 2.26. To a second 100 ml of filtrate add 5 ml of 20% phosphotungstic acid soln, shake thoroly, allow to settle, and filter by decantation. Wash slightly with  $\text{H}_2\text{O}$ , concentrate filtrate with 5 ml of  $\text{H}_2\text{SO}_4$  in Kjeldahl flask, and determine non-protein N as directed under 2.24, 2.25, or 2.26. Deduct non-protein N from N found in first fraction to obtain the N as protein soluble in 1% NaCl soln.  $\text{N} \times 5.7 = \text{protein}$ . Make allowance for N contained in reagents.

**20.35 PROTEIN INSOLUBLE IN POTASSIUM SULFATE SOLUTION AND 70 PER CENT ALCOHOL—TENTATIVE**

Deduct sum of the  $\text{K}_2\text{SO}_4$ -soluble N, 20.33, and the alcohol-soluble N, 20.32, from total organic and ammoniacal N, 20.31, and multiply difference by 5.7.

**20.36 PROTEIN INSOLUBLE IN BARIUM HYDROXIDE—METHYL ALCOHOL MIXTURE (17)—TENTATIVE**

(Flour and reagents should be allowed minimum exposure to air at all times.)

Weigh 8 g of flour into 200 ml flask, preferably sugar flask or one that readily permits thoro mixing of suspension when shaken. Add 0.2 g of freshly powdered  $\text{Ba}(\text{OH})_2$ , follow at once with 50 ml of  $\text{H}_2\text{O}$  ( $\text{CO}_2$ -free), and stopper tightly. Shake immediately to form smooth suspension. Let stand 1 hour at room temp., shaking frequently. Add sufficient 96% methyl alcohol free from acids, aldehydes, and ketones (synthetic methanol preferred) to allow 5 ml of liquid above mark (to correct for volume of flour) when thoroly mixed. Shake vigorously 2 min. After starch settles to bottom, pour supernatant liquid *at once* thru cotton plug, repeating filtrations 2 or 3 times if necessary. Immediately withdraw 50 ml for Kjeldahl N determination, 2.24, 2.25, or 2.26. Do not allow more than 15 min. to elapse from time the methyl alcohol is added to withdrawal of the 50 ml aliquot, because gliadin will begin to precipitate after standing short period of time. To prevent troublesome foaming add 150–200 ml of  $\text{H}_2\text{O}$  to Kjeldahl flask before starting digestion of the alcoholic extract. Convert N to protein by factor 5.7, subtract percentage of protein in extract from percentage of total protein ( $\text{N} \times 5.7$ ) as determined in separate portion of flour, and record difference as percentage of glutenin in flour.

**CRUDE GLUTEN****20.37 Qualitative Test (18)—Tentative**

Place small quantity of flour (ca 1.5 mg) on microscope slide; add a drop of  $\text{H}_2\text{O}$  containing 0.2 g of water-soluble eosin in 1 liter; and mix by means of cover-glass, holding it at first in such a manner that it is raised slightly above slide and taking care that none of flour escapes from beneath it. Finally allow cover-glass to rest on slide and rub it back and forth until gluten has collected into rolls. Carry out operation on white paper so that formation of the gluten rolls can be easily noted. Wheat flour, or other flours containing gluten, show by this treatment a copious quantity of gluten, which absorbs the eosin with avidity, assuming a carmine color. Rye flour and corn flour yield only trace of gluten; buckwheat flour, no appreciable quantity. If flour is coarse or contains considerable quantity of bran elements, as is true of buck-

wheat flour and low-grade wheat flour, make test after bolting, as the bran particles and coarse lumps interfere with formation of gluten rolls.

## 20.38

*Quantitative Method—Tentative*

(Results are approximate)

Weigh 25 g of flour into cup or porcelain mortar; add sufficient tap  $H_2O$  (ca 15 ml) to form firm dough ball; and work into dough with spatula or pestle, taking care that none of material adheres to utensil. Allow dough to stand in  $H_2O$  at room temp. an hour; knead gently in stream of tap  $H_2O$  until starch and all soluble matters are removed. Do this operation, which requires ca 12 min., over bolting cloth. To determine whether or not the gluten is starch-free let 1 or 2 drops of the wash  $H_2O$ , obtained by squeezing the gluten, fall into beaker containing perfectly clear  $H_2O$ . If starch is present, cloudiness appears. Allow gluten thus obtained to stand in  $H_2O$  an hour, press as dry as possible with hand, roll into ball, place in weighed flat-bottomed dish, and weigh as moist gluten. Transfer to oven, dry to constant weight at  $100^\circ$  (ca 24 hours), cool, and weigh as dry gluten; or heat moist gluten at ca  $230^\circ$  15–20 min., or until puffed gluten ball has become firm. Dry to constant weight in drying oven.

## 20.39 WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL (19)—TENTATIVE

Place 20 g of sample in 8 oz. nursing bottle, add 100 ml of  $H_2O$  from pipet, shake bottle to prevent lumping of sample, and add exactly 100 ml more  $H_2O$ . Mix contents of stoppered bottle gently by hand or on slowly revolving wheel 1 hour. (Temp. of  $H_2O$  should not exceed  $30^\circ$ .) Centrifuge to facilitate filtration and filter thru thin pad of ignited asbestos (fine) in Hirsch funnel, using light suction. Determine N in 50 ml of filtrate as directed under 2.24, 2.25, or 2.26, distilling the  $NH_3$  into 20 ml of 0.1 *N* acid. Run blank on reagents. Pipet off 100 ml of above filtrate into 200 ml volumetric flask, add 15 ml of NaCl soln (28 g diluted to 300 ml), fill nearly to mark with alcohol, mix carefully to avoid foaming, cool to room temp., make up to mark with alcohol, mix well, and allow to stand overnight. Pipet off supernatant liquid and filter thru  $18\frac{1}{2}$  cm fluted filter paper. Determine N in 100 ml of filtrate as above. (In order to avoid bumping, it is advisable to add the  $H_2SO_4$  and boil off the alcohol before adding the  $K_2SO_4$  and  $HgO$ .) Subtract value obtained from water-soluble N to obtain water-soluble N precipitable by 40% alcohol.

## 20.40

## LIPOIDS (20)—OFFICIAL

Add 15 ml of alcohol, 70% by volume, to 5 g of flour in 200 ml nursing bottle. Give bottle gentle rotary motion so as to moisten all particles with the alcohol, stopper, and set in water bath kept at  $75-80^\circ$ . Heat 15 min. with frequent mixing by same rotary motion. Add 27 ml of alcohol, stopper bottle, and shake vigorously 2 min. Cool, add 45 ml of ether, and shake well 5 min. (Sample should now be in fine state of division.) Centrifuge just sufficiently to throw solid particles out of suspension but not enough to pack sample too firmly. Decant liquid into 250 ml beaker containing some bits of broken porcelain or glass, and rinse off bottle neck with ether. Re-extract sample with 3 successive 20 ml portions of ether, shake 1 or 2 min. each time, centrifuge, and decant into beaker containing first extract. Evaporate combined ether-alcohol extracts just to dryness on steam bath. Drive off any remaining moisture on sides of beaker by placing in drying oven at  $100^\circ$  5 min. Dissolve dry extract in ca 15 ml of  $CHCl_3$  and filter soln into previously dried and weighed Pt dish thru pledget of cotton packed in stem of funnel. Free with glass

rod any solid extract adhering to beaker and wash all extract from bottom and sides of beaker onto filter with stream of  $\text{CHCl}_3$  from wash bottle. Finally wash funnel and stem tip. Evaporate the  $\text{CHCl}_3$  on steam bath and dry dish and contents in drying oven at  $100^\circ$  to constant weight (75–90 min.). Weigh. Report extract as lipoids.

**20.41 LIPOID PHOSPHORIC ACID ( $\text{P}_2\text{O}_5$ ) (#1)—OFFICIAL**

Dissolve lipoids in 5–10 ml of  $\text{CHCl}_3$ , add 5–10 ml of 4% alcoholic KOH soln, evaporate to dryness on steam bath, and char well in furnace at faint red heat ( $500^\circ$ ). Cover dish with cover-glass, add sufficient  $\text{HNO}_3$  (1+9) to make soln slightly acid, warm on steam bath, and filter. Wash residue and filter well with hot  $\text{H}_2\text{O}$ . Determine  $\text{P}_2\text{O}_5$  in filtrate as directed under 2.9 or 2.12. Report as lipid  $\text{P}_2\text{O}_5$ .

**20.42 UNSAPONIFIABLE RESIDUE—OFFICIAL.—See 20.122**

**20.43 EXTRACT SOLUBLE IN COLD WATER (#2)—OFFICIAL**

Weigh 20 g of flour into 500 ml Erlenmeyer flask and add gradually 200 ml of  $\text{H}_2\text{O}$  at temp. of ca  $0^\circ$ . Shake vigorously when ca 50 ml of  $\text{H}_2\text{O}$  has been added and continue shaking during addition of remaining  $\text{H}_2\text{O}$ . Allow mixture to stand at  $0^\circ$  40 min., shaking occasionally. Filter rapidly, returning first runnings to filter, until clear filtrate is obtained. Pipet 20 ml of clear filtrate into weighed dish, evaporate to dryness on steam bath, and dry in vacuum oven at ca  $100^\circ$  for 30 min. periods until constant weight is attained.

**STARCH—TENTATIVE**

*Gravimetric Method* (#3)

(Applicable to uncooked cereal products)

**20.44 REAGENT**

*Hydrochloric acid.*—Mix ca equal volumes of HCl and  $\text{H}_2\text{O}$  and adjust by titration so that 100 ml of this soln contains 20.5–21.0 g of HCl.

**20.45 DETERMINATION**

Weigh accurately sufficient finely ground sample (should readily pass thru 20-mesh sieve) to represent 0.5–1.0 g of starch. Transfer to funnel fitted with 9 cm S & S No. 589 white ribbon or Whatman No. 40 filter paper and extract by nearly filling the filter 4 times with ethyl ether; likewise extract with alcohol (70% by volume) and with  $\text{H}_2\text{O}$ . Allow to drain 1 hour uncovered. Transfer drained filter and contents to 50 ml beaker. In next step use stirring rod having flattened button-like end 15 mm in diam., and (very important) tamp with a twisting motion during time specified in order to get filter paper completely disintegrated and thus insure complete suspension of starch in the HCl soln without hydrolysis of any of it. Complete maceration while there is small amount of HCl present and whole contents are rather thick paste. (If this optimum condition is obtained practically duplicate results will follow.) Add the HCl reagent at  $15^\circ$  to beaker containing sample, using a fast delivering 10 ml Mohr pipet with 1 ml marked off at lower end with heavy pencil marks. Keep acid supply on bench, but do not allow it to get above  $18^\circ$ .

Proceed as follows, adding the HCl in quantities given: Add 1 ml, tamp 1 min.; add 1 ml, tamp 2 min.; add 1 ml, tamp 2 min.; add 1 ml, tamp 1 min.; add 1 ml, tamp 1 min.; add 1 ml, tamp 1 min.; add 1 ml, tamp 1 min.

Fill beaker half full with the acid and stir 30 sec. Fill beaker  $\frac{3}{4}$  full and stir 30 sec.

(In 10 min. by this treatment paper should be completely disintegrated and in smooth state of suspension; tamping should be continued vigorously during this time, and as little time as possible should be spent adding the acid.) Immediately transfer to 100 ml wide-mouthed volumetric flask, rinsing out beaker with the HCl; carefully make to volume with the HCl reagent and add 0.5 ml for volume of filter paper (this step requires 2 min.). Shake stoppered flask vigorously 5 min., and allow to stand 5 min. in beaker of H<sub>2</sub>O at 20°. Shake twice and filter immediately into 250 ml suction flask thru small Büchner funnel (41 mm in diam.) fitted with thin layer of asbestos and filled half full with dry, fluffy asbestos. (Filtration requires 1 min. only.) Immediately pipet 50 ml of filtrate into 200 ml beaker (tall form) containing 115 ml of alcohol. (Quantity of starch finally weighed will then vary from 0.25 to 0.5 g. Time consumed from initial addition of acid is 24 min.) Allow pipet to drain completely and then stir with whipping motion 1 min. to flocculate precipitated starch. Wash down sides of beaker with 70% alcohol. Allow to stand 3–4 min., until nearly all precipitate has settled, and then carefully decant supernatant liquid, which is somewhat turbid, so that little or no precipitate passes into weighed Gooch crucible, which has been fitted with thin pad of ignited asbestos and is half filled with fluffy ignited asbestos. Wash precipitate and filter by decantation, using successively two 40 ml portions of 70% alcohol (by volume), then 4 times, using ca 30 ml portions of alcohol, each time breaking up precipitate by rapid stirring and allowing precipitate to settle before decantation. After each stirring rinse sides of beaker with small stream of alcohol to prevent starch from drying and sticking. Finally transfer starch completely by means of jet of alcohol and wash sides of Gooch and precipitate with a little of the alcohol. (All these filtrations are very fast.) Dry crucible and contents uncovered 2 hours at 130°; cover crucible immediately and place in desiccator charged with P<sub>2</sub>O<sub>5</sub>, fresh H<sub>2</sub>SO<sub>4</sub>, or freshly ignited CaO; cool 10 min. and weigh. Multiply result by 2 and report as starch.

*Caution:* To obtain satisfactory results, these directions must be followed carefully in every detail. As the steps are timed it is essential to learn procedure so that no time will be lost in following it thru. Arrange everything needed in determination before the HCl is added to sample.

#### *Polarimetric Method (24)*

##### 20.46

##### REAGENT

*Calcium chloride soln.*—Dissolve 2 parts of crystalline CaCl<sub>2</sub>·6H<sub>2</sub>O in one part of H<sub>2</sub>O and adjust to density of 1.30 at 20°. (This soln contains ca 33% of CaCl<sub>2</sub>.) Make faintly pink to phenolphthalein by adding 0.1 N NaOH. (Anhydrous CaCl<sub>2</sub> may be used, but it is usually alkaline and requires the addition of acid to bring it to correct pH.)

##### 20.47

##### DETERMINATION

Grind sample finely (100-mesh if possible) and weigh 2.0–2.5 g into 50 ml round-bottomed centrifuge tube with lip. Wash with ether to remove fat, then with 10 ml of ca 65% by weight alcohol (d<sub>20</sub> 0.88) and stir thoroly with glass rod. Centrifuge (1) and pour off soln. Repeat washing until 60 ml of wash liquid has been used, stirring each time with same rod.

Stir residue with 10 ml of H<sub>2</sub>O and pour into 200–250 ml Erlenmeyer flask. Complete transfer by washing out with total of 60 ml of the CaCl<sub>2</sub> soln containing 2 ml of 0.8% acetic acid. Transfer rod to flask and bring mixture to boiling quickly over wire gauze, stirring frequently. Boil briskly for 15–17 min., taking precautions

to prevent burning and foaming (2). Rub particles on sides of flask down with rod from time to time.

Cool soln quickly in running  $H_2O$  and pour into 100 ml volumetric flask, rinsing thoroly with the  $CaCl_2$  soln from wash-bottle with medium jet. (In making up to mark, it is permissible to destroy froth by adding one drop of alcohol.)

After thoroly mixing sample pour ca 10 ml of soln onto fluted filter (Whatman No. 42 or 44), wetting paper completely. Allow filtrate to run dry and discard. Resume filtration, using dry receiver, and collect 40–50 ml (3).

Polarize liquid in 10 cm tube, taking 2 sets of 10 readings each. (Averages of two sets should agree within  $0.006^\circ$ .) Calculate starch content as follows:

$$\text{Percentage starch (4)} = \frac{100 \times A \times 100}{1 \times 200 \times S} = \frac{50 \times A}{S}, \text{ where } A \text{ is observed angular}$$

rotation and  $S$  is weight of sample.

#### NOTES:

(1) If no centrifuge is available wash samples on filter paper, using Pt cone and slight suction.

(2) An Argand burner with a thin asbestos gauze will diminish foaming.

(3) For filtering aids, Celite with Pyrex glass filters or Hirsch-type funnel with asbestos and suction are recommended.

(4) 200 is arbitrarily taken as specific rotation for all starches until a better figure is worked out for individual starches. If 200 mm tube and saccharimeter are used, and 2 g of sample is weighed out and mixture is made up to 100 ml,  $^\circ V \times 4.3225 = \text{per cent starch}$ .

#### CHLORINE IN FAT OF FLOUR

##### 20.48 Qualitative Test (Chlorine-Bleached Flours)—Tentative

Extract 30 g of flour with 50 ml of petroleum benzine and allow solvent to evaporate. (Small quantity of oil remains.) Heat piece of Cu wire in colorless gas flame until it is black and no longer colors flame green. Dip hot end of wire into oil and again bring into flame. If Cl or Br has been used as bleaching agent, green or blue coloration is produced.

#### Quantitative Method (25)—Official

##### 20.49

##### EXTRACTION OF FAT

Weigh 500 g of the flour into 2 liter flask. Add 700 ml of petroleum benzine and shake at 5 min. intervals for 30 min. Filter thru Büchner funnel, pressing flour to obtain as much solvent as practicable. Transfer the benzine extract to large beaker and evaporate on steam bath to ca 10 ml. Filter into container thru small funnel containing pledget of cotton packed firmly in stem. (Filtrate must be clear and free from flour.)

##### 20.50

##### DETERMINATION

Heat porcelain crucible of ca 90 ml capacity containing 10 g of fusion mixture (138 g of anhydrous  $K_2CO_3$ , 106 g of anhydrous  $Na_2CO_3$ , and 75 g of powdered  $KNO_3$ ) 30 min. in  $100^\circ$  oven; dry in desiccator and weigh. Transfer the filtered 10 ml of benzine extract to the crucible, using petroleum benzine for rinsing. Evaporate petroleum benzine on steam bath and dry fat in  $100^\circ$  oven for 30 min. Cool, and determine weight of fat by difference. Add to the crucible 5 g more of the fusion mixture and spread evenly. Burn to white ash in muffle at temp. of  $525^\circ$  (ca 1 hour).

Add 25 ml of hot  $H_2O$  to mixture and transfer with small quantity of hot  $H_2O$  to 200 ml tall-form beaker or beaker flask. Add  $HNO_3$  cautiously until soln is slightly



acid to litmus paper. Add 25 ml more  $\text{HNO}_3$ . Add 5 ml of 0.3  $N$   $\text{AgNO}_3$  soln. Boil 5 min. in hood. Cool to room temp. Filter thru 9 cm No. 1 Whatman filter paper, or similar Cl-free filter paper. Use 1%  $\text{HNO}_3$  soln for rinsing. Proceed with the digestion as directed under 12.46, beginning, "Place filter paper and contents in Kjeldahl flask." After digestion use 175 ml of  $\text{H}_2\text{O}$ . Run a blank on the reagents. Report Cl as mg/1 g of fat.

#### NITRITE NITROGEN—TENTATIVE

##### 20.51

##### REAGENTS

(a) *Sulfanilic acid soln.*—Dissolve 0.5 g of sulfanilic acid in 150 ml of 20% acetic acid.

(b) *Alpha-naphthylamine hydrochloride soln.*—Dissolve, by heating, 0.2 g of the salt in 150 ml of 20% acetic acid.

(c) *Standard nitrite soln.*—Dissolve 0.1097 g of dry  $\text{AgNO}_2$  in ca 20 ml of hot  $\text{H}_2\text{O}$ , add 0.10 g of  $\text{NaCl}$ , shake until the  $\text{AgCl}$  flocculates, and dilute to 1 liter. Draw off 10 ml of the clear soln and dilute to 1 liter. 1 ml of this nitrite soln = 0.0001 mg of N.

The  $\text{AgNO}_2$  may be prepared as follows: To cold soln of ca 2 g of  $\text{NaNO}_2$  or  $\text{KNO}_2$  in 50 ml of  $\text{H}_2\text{O}$ , add soln of  $\text{AgNO}_3$  so long as precipitate forms. Decant liquid and thoroly wash precipitate with cold  $\text{H}_2\text{O}$ . Crystallize from boiling  $\text{H}_2\text{O}$  and dry crystals in dark at ordinary temp. (preferably in vacuum).

##### 20.52

##### DETERMINATION

(a) Select a series of 100 ml volumetric flasks of uniform dimensions and color and place 2 g of high-grade, nitrite-free flour in each flask; add ca 70 ml of nitrite-free  $\text{H}_2\text{O}$  and shake until flour is thoroly moistened. Add to flasks varying quantities of the standard  $\text{NaNO}_2$  soln, so that a series of comparison standards will be obtained having a range covering probable nitrite content of unknown sample. Reserve one flask for blank test. In order to avoid making a large series of standards it is well to make preliminary test to ascertain approximate nitrite content of unknown. If quantity of nitrite present is small, the nitrite soln in flasks may be increased by 0.4 ml each. If bleaching is excessive, 1 g of flour may be used thruout, or standards may be given a wider variation in nitrite content.

To each of 2 similar flasks add 2 g of the unknown flour and 90 ml of  $\text{H}_2\text{O}$ , shake thoroly, digest all the flasks, including blank, in water bath at  $40^\circ$  at least 15 min., and add 2 ml each of the sulfanilic acid and alpha-naphthylamine hydrochloride solns to each flask, shaking mixture after addition of each reagent. Continue digestion at  $40^\circ$  for an additional 20 min. (The color must be developed in all the flasks under conditions as nearly uniform as possible.) Make up to marks with nitrite-free  $\text{H}_2\text{O}$  and compare unknown with series of standards. (This may be done in large, white-enamelled pan, effect of turbidity due to flour being minimized by white background.) Solns should be allowed to subside and should not be shaken during comparison. Or,

(b) Weigh 20 g of the flour into 500 ml Erlenmeyer flask; add 200 ml of nitrite-free  $\text{H}_2\text{O}$ , previously warmed to  $40^\circ$ ; and close flask with rubber stopper. Shake vigorously 5 min. and digest 1 hour in water bath, keeping temp. of liquid in flask at  $40^\circ$  and shaking at 10 min. intervals. Finally filter thru a nitrite-free filter. Return first runnings to filter until clear filtrate is obtained. Pipet 50 ml of filtrate and 50 ml of standard nitrite soln into small flasks; add to each 50 ml of  $\text{H}_2\text{O}$  and 2 ml each of the sulfanilic acid and alpha-naphthylamine hydrochloride solns; shake; and allow to stand 1 hour to bring out color. Compare two solns in colorimeter. Divide height of column of standard soln by that of the soln of sample to obtain parts of nitrite N (free and combined) per million of flour.

## 20.53

## BENZOYL PEROXIDE BLEACH IN FLOUR (86)—TENTATIVE

Add a mixture of 125 g of flour, 100 g of NaCl, and 80 g of  $\text{CaCl}_2$  (dried) to short-necked 800 ml Kjeldahl flask that contains 250 ml of  $\text{H}_2\text{O}$  and 30 ml of HCl, and shake vigorously. Immediately connect for steam distillation and distil 325 ml as rapidly as possible after initial foaming has ceased. Saturate distillate with 100 g of NaCl, transfer to 500 ml separator, and extract with 50 ml of ether. Again extract NaCl soln with 50 ml of ether and discard NaCl soln. Pour ether from two separators into large, flat crystallizing dish and evaporate at room temp. with aid of electric fan. Dissolve residue in 5 ml of acetone, add 7 ml of 2 N NaOH, and transfer to 150 ml beaker. Rinse crystallizing dish into beaker with 35–40 ml of  $\text{H}_2\text{O}$ . Heat over flame, carefully at first, then boil ca 20 min. until all acetone is removed. Add  $\text{H}_2\text{O}$  occasionally to keep volume ca constant. While hot transfer to separator and add 25 ml of amyl alcohol. Pour lower layer into 250 ml separator and extract again with 20 ml of amyl alcohol. Pour lower layer into 250 ml casserole. Combine amyl alcohol solns, add equal volume of petroleum benzine, extract 3 times with 5 ml of  $\text{H}_2\text{O}$ , and add to aqueous soln in casserole. Discard the amyl alcohol-benzine soln. Add 2 ml of superoxol (30%  $\text{H}_2\text{O}_2$ ) to aqueous soln. Bring to boiling slowly and boil until foaming ceases. Cool. Make acid to litmus with  $\text{H}_2\text{SO}_4$  (1+1). Pour into small separator. Cool, and extract twice with 20 ml of mixture of equal parts of ether and petroleum benzine. Pour combined extracts into large test tube, add 2 ml of 2 N NaOH, stopper tube, and shake. Place thread in tube to insure even boiling and evaporate, slowly at first, by holding over steam. Place tube in vigorously boiling saturated NaCl soln. Add drop of superoxol and when foaming ceases add another drop. Continue adding a drop at a time until soln is almost colorless. Add a drop or two of  $\text{H}_2\text{O}$  occasionally if evaporation is too rapid. Evaporate completely to dryness and heat at  $100^\circ$  in vacuum ca 30 min. Cool, and add 0.3 g of  $\text{KNO}_3$  and 3 ml of  $\text{H}_2\text{SO}_4$ . Heat in boiling water bath 20 min., taking care to get all solid material into soln (stirring rod is essential). Cool the tube in cold  $\text{H}_2\text{O}$  and add 6 ml of  $\text{H}_2\text{O}$  with stirring. When cool, add 15 ml of  $\text{NH}_4\text{OH}$  slowly with continuous stirring to keep soln cool. Add 2 ml of 6% hydroxylamine hydrochloride soln and place in  $65^\circ$  water bath 5–6 min., stirring occasionally. Cool in cold  $\text{H}_2\text{O}$ , filter into another similar tube, and observe color of filtrate. Red color indicates presence of benzoic acid.

To make this method semiquantitative proceed as follows:

Prepare series of standard tubes containing 0.2–1.5 mg of benzoic acid in ether soln (1 mg to 1 ml). Add 2 ml of 2 N NaOH to each. Mix by shaking and proceed exactly as with sample, starting, "place tube in vigorously boiling saturated NaCl soln." Comparison of sample with standards familiarizes the analyst with color to be expected and offers approximate estimation of amount of benzoic acid recovered. For calculation of approximate amount of benzoic acid in p.p.m. multiply sample reading in mg by 32. This factor is based on 125 g sample and minimum recovery of 25% benzoic acid.

## 20.54

## GASOLINE COLOR VALUE (87)—TENTATIVE

Place 20 g of flour in wide-mouth, glass-stoppered 120 ml bottle and add 100 ml of colorless gasoline. Stopper tightly and shake vigorously 5 min. Allow to stand 16 hours, shake again a few seconds until flour has been loosened from bottom of bottle and thoroly mixed with gasoline, then filter immediately thru dry 11 cm paper into Erlenmeyer flask, keeping funnel covered with watch-glass to prevent evaporation. In order to secure clear filtrate, allow a certain quantity of the flour to pass over into filter, and pass first portion of filtrate thru a second time. (It will be found

convenient to fit filter paper to funnel by means of H<sub>2</sub>O and to dry thoroly either by standing overnight in well-ventilated place or by heating.)

Determine color value of clear gasoline soln in Schreiner or similar colorimeter, using for comparison 0.005% K<sub>2</sub>CrO<sub>4</sub> soln. This soln corresponds to a gasoline number of 1.0 and is conveniently prepared by diluting 10 ml of a 0.5% soln to 1 liter. Adjust colorimeter tube containing gasoline soln to read 50 mm, and raise or lower tube containing standard K<sub>2</sub>CrO<sub>4</sub> soln until shades of yellow in both tubes match. Reading of K<sub>2</sub>CrO<sub>4</sub> soln ÷ reading of gasoline soln = gasoline color value. Color value may also be determined in Nessler tubes by using for comparison K<sub>2</sub>CrO<sub>4</sub> solns of various dilutions prepared from 0.5% soln and filling tubes in all cases to height of 50 mm.

#### CAROTENE—TENTATIVE (§8)

##### 20.55

##### STANDARDIZATION OF NEUTRAL WEDGE PHOTOMETER

Dissolve 100 mg of a natural mixture of alpha and beta carotene in 5–6 ml of CS<sub>2</sub>, add 35–40 ml of absolute alcohol, cool in refrigerator ca 1 hour to insure maximum crystallization, and filter on *hard* filter paper. Dissolve the carotene crystals in 5–6 ml of CS<sub>2</sub>, add 40 ml of petroleum benzine, cool in refrigerator as before, filter on *hard* filter paper, and dry crystals in vacuum desiccator for 1 hour.

Weigh accurately 20 mg of purified crystals and wash with 20 ml of absolute ether into 1000 ml graduated glass-stoppered flask. Continue to wash with petroleum benzine, and make to volume by adding petroleum benzine as soon as the carotene completely dissolves. Designate this as stock soln.

Make up eight concentrations by adding the following amounts of this stock soln to a 250 ml graduated flask: 1.25, 2.50, 3.75, 5.00, 6.25, 7.5, 8.75, and 10.00 ml. Make to volume with petroleum benzine. These dilutions represent concentrations of 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, and 0.80 mg/liter. Read these solns in a 4" cell, using filter No. 44 in the photometer eyepiece. (The cell, filled with the solvent, should read zero on the photometer scale.) Make 10 readings on each soln and plot average reading against concentration. The line of best fit for the data is obtained by the method of least squares. In application of this method let  $x$  represent the scale reading and  $y$  the concentration in mg/liter. Substitute in the following expressions for the values of  $a$  and  $b$  to give the equation  $y = a + bx$ :

$$b = \frac{\Sigma xy - nM_xM_y}{\Sigma(x)^2 - n(M_x)^2} \quad \text{and} \quad a = M_y - bM_x.$$

Do the standardization the same day the stock soln is prepared, and standardize neutral wedge photometer. Spectrophotometer or photoelectric colorimeter may be used.

##### 20.56

##### PREPARATION OF SAMPLE

Grind macaroni and noodles to as near flour fineness as possible in the ordinary coffee-type mill. (No difficulty occurs with products containing egg, but plain macaroni products require several grindings.) Take care not to set the mill too tight, as the heat generated may be sufficient to damage the pigments.

##### 20.57

##### REAGENTS

- (a) *Alcoholic potassium hydroxide soln.*—10 g of KOH/100 ml of alcohol.
- (b) *Methyl alcohol.*—92% . 8 ml of H<sub>2</sub>O + 92 ml of absolute methyl alcohol.

## 20.58

## APPARATUS

- (a) *Separators*.—125 and 250 ml.
- (b) *Distillation flask*.—250 ml.
- (c) *Neutral wedge photometer, spectrophotometer, or photoelectric colorimeter, and absorption cells*.—2" and 4".

## 20.59

## DETERMINATION

Weigh 20 g of flour, semolina, or macaroni, or 10 g of egg noodles, or 2 g of egg yolk, into 125 ml Erlenmeyer flask, add 50 ml of the alcoholic KOH soln, and heat on steam bath for 30 min. with flask fitted to a reflux condenser. Rotate flask occasionally during this time, but be as careful as possible to keep sample from collecting on sides of flask. Remove flask, and cool to room temp. Filter soln thru Büchner-type fritted glass filter (11G3), using suction, transferring all material with a little alcohol from wash bottle. Turn off suction, rinse out flask with 25 ml of ether, and pour rinsings onto glass filter, stirring material with rod to allow ether to come in contact with all portions. Filter off and repeat this procedure twice more. Transfer filtrate to a 250 ml glass-stoppered separator, rinsing with ca 25 ml of ether. Disregard any resinous material. Add 175 ml of cold tap H<sub>2</sub>O, carefully invert, and rotate several times. When the aqueous alcohol and ether layers have separated, remove lower aqueous alcohol layer and extract this layer again with 25 ml of ethyl ether. Discard lower layer. Add the ether to original ether soln. Wash the ether by pouring 50 ml of tap H<sub>2</sub>O thru it. After separation of layers, withdraw H<sub>2</sub>O layer and discard it. Add to the ether soln 50 ml of petroleum benzine and wash 5 times with 50 ml of tap H<sub>2</sub>O, shaking gently by inverting and rotating the separator. Discard all H<sub>2</sub>O layers. (Slight emulsions may occur but can be disregarded.)

Transfer the ether-petroleum benzine mixture to a 250 ml distillation flask, and place flask in beaker of H<sub>2</sub>O at 45–50°. Stopper flask, connect side arm with vacuum, and concentrate to ca 5 ml to remove the ether. Filter thru paper two-thirds filled with anhydrous Na<sub>2</sub>SO<sub>4</sub>, into 100 ml volumetric flask. Make to volume with petroleum benzine that has been passed portion-wise thru the filter and Na<sub>2</sub>SO<sub>4</sub>. (If a total color determination is desired, fill the 4" absorption cell with a pipet, since none of the soln must be lost during the filling, and read in the neutral wedge photometer, using filter No. 44. Return soln in cell to flask.) Transfer soln to 125 ml separator, rinsing with a little petroleum benzine. Add 15 ml of 92% methyl alcohol, shake on mechanical shaker 10 min., or 2–3 min. by hand, remove, and allow separator to stand in upright position a minute until alcohol and ether layers separate. Decant lower layer containing xanthophyll and repeat extraction 5 more times, or until the aqueous methyl alcohol layer is colorless. (It is necessary to examine the final methyl alcohol layer in a test tube over a white background to be sure it is colorless; 6 extractions are usually sufficient on flour and macaroni, but noodles with high egg content may require 10 extractions.)

Wash with 25 ml of H<sub>2</sub>O, inverting the separator several times; decant, and discard the H<sub>2</sub>O layer. Repeat twice more. After the last washing pour the petroleum benzine layer into the 250 ml distillation flask. Place flask in beaker of H<sub>2</sub>O at 45–50°, stopper flask, and concentrate the petroleum benzine by vacuum as before. Concentrate to 10–15 ml and filter thru dry filter paper two-thirds filled with anhydrous Na<sub>2</sub>SO<sub>4</sub> into 25 or 50 ml volumetric flask, depending on concentration of carotene. Wash filter paper with petroleum benzine in making to volume. Mix contents and read concentration in a 4" absorption cell in neutral wedge photometer, using filter No. 44. From the standardization curve, read off concentration of

carotene. Divide by 4 or 2, depending on volume adjustment, to obtain concentration of carotene in 100 ml. Multiply by 5 to convert to p.p.m. in sample if 20 g sample was used, and by 10 for 10 g sample, etc.

Total color value calculated as carotene may be obtained, when desired, from the same standardization curve, since carotene and xanthophyll show nearly identical absorption with the No. 44 filter. It represents ca the amount of xanthophyll and carotene. Dilution for total color is already 100 ml. Therefore to convert to p.p.m. in sample multiply by 5 if 20 g sample was used, and by 10 for 10 g sample, etc.

#### DETECTION OF RYE FLOUR IN WHEAT FLOUR (29)

##### 20.60

##### *Chloroform Test—Tentative*

(a) *In ordinary flours.*—To 10 g of flour in test tube, add 20 ml of  $\text{CHCl}_3$ , stopper tube, and shake well. Allow tube to stand in vertical position until heavier particles have settled out, preferably overnight. If rye is present, sediment in tube will be of greenish or bluish tint. Wheat gives yellowish sediment.

Make comparisons with wheat and rye flours of known purity and with mixtures of varying proportions, such as 5, 10 and 15%, etc., of rye.

(b) *In phosphated flours.*—Treat flours containing phosphate or leavening agents with  $\text{CCl}_4$  in separator to remove added salts. After removing sediment of salt from the separator collect flour on filter, transfer to test tube, and treat with  $\text{CHCl}_3$ .

#### DIASTATIC ACTIVITY OF FLOUR (30)—OFFICIAL

##### 20.61

##### REAGENTS

(a) *Buffer soln.*—Make up 3 ml of acetic acid and 4.1 g of anhydrous Na acetate to 1 liter with  $\text{H}_2\text{O}$ . The pH of this soln is 4.6–4.8.

(b) *Alkaline ferricyanide soln.*—16.5 g of pure dry  $\text{K}_3\text{Fe}(\text{CN})_6$  and 22 g of anhydrous  $\text{Na}_2\text{CO}_3$  in 1 liter of  $\text{H}_2\text{O}$ . The  $\text{K}_3\text{Fe}(\text{CN})_6$  soln is 0.05 N. It maintains its strength for long period of time if kept in dark glass bottle away from light. (The best C. P. grade of this salt purchased on market may ordinarily be depended upon to be free from moisture and impurities.)

(c) *Sodium thiosulfate soln.*—0.05 N. 12.41 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ /liter. Select only clear crystals from best C.P. grade. If redistilled  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  (second distillation being made after addition of small quantity of alkaline  $\text{KMnO}_4$  soln to first distillation, to destroy all traces of organic matter) is used in making up this soln, it will retain its normality a long time, whereas with ordinary distilled  $\text{H}_2\text{O}$  it is likely to deteriorate slowly on standing. Check the  $\text{Na}_2\text{S}_2\text{O}_3$  against the soln as follows: To 10 ml of the  $\text{K}_3\text{Fe}(\text{CN})_6$  soln add 25 ml of the acetic acid reagent (d) followed by 1 ml of 50% KI and 2 ml of soluble starch soln. Titrate with the  $\text{Na}_2\text{S}_2\text{O}_3$  soln. (It should require exactly 10 ml of the  $\text{Na}_2\text{S}_2\text{O}_3$  soln to completely discharge blue starch-iodine color.) Standardize the  $\text{Na}_2\text{S}_2\text{O}_3$  soln against pure I if necessary.

(d) *Acetic acid soln.*—200 ml of acetic acid, 70 g of KCl, and 20 g of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  per liter.

(e) *Potassium iodide soln.*—50% soln of KI. Add 1 drop of NaOH (1+1) for each 100 ml of soln to prevent or substantially delay deterioration of the soln (with liberation of I) on standing, which will otherwise occur. (Soln must be colorless.)

(f) *Soluble starch soln.*—1% of soluble starch in 30% NaCl soln. Prepare soluble starch suspension and pour slowly into boiling  $\text{H}_2\text{O}$ . Add NaCl and make to volume. (Soln should be transparent and colorless.)

## 20.62

## DETERMINATION

(Total maltose after diastasis for 1 hour)

Introduce 5 g of flour and teaspoonful of ignited quartz sand into 100 or 125 ml Erlenmeyer flask, and mix by rotating flask: Add 46 ml of buffer soln, and again mix by rotating flask until all flour is in suspension. (Flask and all ingredients should be *individually* brought to 30° before being mixed together.) Digest 1 hour at 30°, preferably in an accurately controlled water thermostat, shaking flask (by rotation) every 15 min. At end of the hour add 2 ml of  $H_2SO_4$  ( $3.58 \pm 0.05 N$ , ca 1+9), and mix thoroly. Add 2 ml of 12% Na tungstate soln, mix, and let stand 1–2 min. Filter thru paper (No. 4 Whatman or its equivalent), discarding first 8 or 10 drops, and pipet 5 ml of filtered extract into test tube of ca 50 ml capacity (18–20 mm diam.). Pipet exactly 10 ml of the  $K_3Fe(CN)_6$  soln into the 5 ml of extract in test tube, and immerse test tube in vigorously boiling water bath. Have surface of liquid in test tube 3–4 cm below surface of boiling  $H_2O$ . (The delay between filtering of extract and treatment in boiling water bath should not be more than 15–20 min. Further delay may cause slight error due to sucrose hydrolysis in the acid soln.) Allow test tube to remain in boiling water bath *exactly* 20 min. Cool test tube and its contents under running  $H_2O$ , and pour at once into 100 or 125 ml Erlenmeyer flask. Rinse out test tube with 25 ml of the acetic acid soln, and add to contents of Erlenmeyer flask, with thoro mixing. Add 1 ml of the KI soln followed by 2 ml of the starch soln, and mix thoroly. Titrate with 0.05  $N$  Na thiosulfate to the complete disappearance of blue color (10 ml buret is recommended). Subtract number of ml

## 20.63

Maltose Conversion Table\*

0.05 $N$ FERRI-CYANIDE REDUCED	MALTOSE EQUIVALENT	0.05 $N$ FERRI-CYANIDE REDUCED	MALTOSE EQUIVALENT	0.05 $N$ FERRI-CYANIDE REDUCED	MALTOSE EQUIVALENT	0.05 $N$ FERRI-CYANIDE REDUCED	MALTOSE EQUIVALENT
<i>ml</i>	<i>mg</i>	<i>ml</i>	<i>mg</i>	<i>ml</i>	<i>mg</i>	<i>ml</i>	<i>mg</i>
0.1	0.2	2.6	4.2	5.1	8.3	7.6	12.3
0.2	0.3	2.7	4.4	5.2	8.4	7.7	12.5
0.3	0.5	2.8	4.5	5.3	8.6	7.8	12.7
0.4	0.6	2.9	4.7	5.4	8.7	7.9	12.9
0.5	0.8	3.0	4.9	5.5	8.9	8.0	13.0
0.6	1.0	3.1	5.0	5.6	9.1	8.1	13.2
0.7	1.1	3.2	5.2	5.7	9.2	8.2	13.4
0.8	1.3	3.3	5.3	5.8	9.4	8.3	13.5
0.9	1.5	3.4	5.5	5.9	9.6	8.4	13.7
1.0	1.6	3.5	5.7	6.0	9.7	8.5	13.9
1.1	1.8	3.6	5.8	6.1	9.9	8.6	14.0
1.2	1.9	3.7	6.0	6.2	10.0	8.7	14.2
1.3	2.1	3.8	6.2	6.3	10.2	8.8	14.4
1.4	2.3	3.9	6.3	6.4	10.4	8.9	14.6
1.5	2.4	4.0	6.5	6.5	10.5	9.0	14.8
1.6	2.6	4.1	6.6	6.6	10.7	9.1	15.0
1.7	2.8	4.2	6.8	6.7	10.9	9.2	15.2
1.8	2.9	4.3	7.0	6.8	11.0	9.3	15.4
1.9	3.1	4.4	7.1	6.9	11.2	9.4	15.6
2.0	3.2	4.5	7.3	7.0	11.3	9.5	15.9
2.1	3.4	4.6	7.5	7.1	11.5	9.6	16.1
2.2	3.6	4.7	7.6	7.2	11.7	9.7	16.5
2.3	3.7	4.8	7.8	7.3	11.8	9.8	17.0
2.4	3.9	4.9	7.9	7.4	12.0	9.9	—
2.5	4.1	5.0	8.1	7.5	12.2	10.0	—

\* Prepared by applying the specified procedure to standard solns of pure maltose and using all reagents in the quantities and volumes precisely as used for flour extracts.

of 0.05  $N$   $Na_2S_2O_3$  used in titration from 10, which gives ml of 0.05  $N$   $K_3Fe(CN)_6$  reduced to ferrocyanide by reducing sugars in flour extract. This value represents a definite quantity of maltose, which may be ascertained by consulting table, 20.63. When 5 ml of flour extract is used, as herein specified, it is necessary merely to multiply mg of maltose by 20 to obtain mg of maltose/10 g of flour in 1 hour's diastasis. This is the value that is recorded and reported as measure of diastatic value of flour in question.

The foregoing specifications may be used with all ordinary flours whose values for mg of maltose produced by 10 g of flour in 1 hour will seldom, if ever, exceed 350. For material giving higher values, such as products from malted or sprouted grain, use smaller portions of extract, i.e., 1, 2, or 3 ml instead of 5 ml. In such cases, however, add enough distilled  $H_2O$  to make up difference, and use different factor for converting results into mg of maltose/10 g of flour. Thus, when 2 ml of extract is used, multiply value obtained from table by 50 instead of 20. If material in test tubes is colorless instead of yellow, after treatment in the boiling water bath, and gives no blue color upon addition of  $KI$ , it is apparent that there was more than enough maltose to reduce all the  $K_3Fe(CN)_6$ , and determination must be repeated with smaller quantity of extract.

## 20.64

### BLANK DETERMINATION

A blank determination, designed to indicate quantity of reducing sugar originally present in the flour—value for which presumably should be deducted from total maltose value after 1 hour's diastasis—has been generally regarded as an essential step in the estimation of flour diastatic activity. This operation, however, is ordinarily unnecessary when dealing with flour milled from *sound* wheat, because quantity of reducing sugars originally present as such is so small and so nearly constant that it may be disregarded for all practical purposes. The blank determination may therefore be conveniently omitted in ordinary routine testing. It need be used only when there is occasion to doubt soundness of the wheat, or in cases where there is known to have been an appreciable quantity of frosted, sprouted, heat-damaged, or otherwise unsound kernels in the wheat from which the flour was milled.

To make blank determination, proceed as follows: Add to 5 g of flour and a teaspoonful of quartz sand in 100 or 125 ml Erlenmeyer flask 48 ml of 0.4% (by volume)  $H_2SO_4$  (preferably pre-cooled to ice-water temp.). Shake mixture thoroly, allow to stand 2 min., and filter thru No. 4 Whatman (or its equivalent) paper. Using 5 ml of clear filtrate, proceed according to 20.62.

### APPARENT VISCOSITY OF ACIDULATED FLOUR-WATER SUSPENSION

*By MacMichael Viscosimeter (31)—Official*

## 20.65

### ADJUSTMENT OF MACHINE

- (a) Use a No. 30 MacMichael viscosimeter wire.
- (b) Have diameter of disk plunger 2.375",  $\pm 0.01$ ".
- (c) Adjust machine so that clearance between bottom of disk and inner surface of bottom of bowl is 0.25",  $\pm 0.005$ ". Check this clearance carefully with depth gage reading in 0.001".
- (d) Use viscosimeter bowl having diameter of ca 7 cm (depth of bowl will vary according to age of machine).
- (e) Adjust regulating device to permit speed of exactly 12 r.p.m., and check carefully and frequently with stop-watch, because as motor warms up machine will have tendency to increase its speed.

(f) Adjust machine and keep it level, and when bob is placed see that it is riding freely and not touching sides of guide.

(g) Adjust dial so that when it comes to rest pointer is on zero mark.

**20.66****PREPARATION OF LACTIC ACID**

Add to concentrated lactic acid approximately right proportion of  $H_2O$  to give slightly stronger soln than normal. Reflux this soln 3 hours, cool, and by addition of  $H_2O$  adjust to normal. Or proceed as follows: Use enough concentrated lactic acid to prepare soln ca 0.85 *N* when standardized with 0.1 *N* NaOH. Transfer this soln to an Erlenmeyer flask fitted with air condenser to prevent undue evaporation of  $H_2O$ , and heat at temp. of 80° for 24 hours (soln will have increased in strength to 1.183 *N*). Adjust to exact normality with  $H_2O$ .

**20.67****PREPARATION OF FLOUR-WATER SUSPENSION**

In clean, dry, 500 ml Erlenmeyer flask, place 20 g of flour (15% moisture basis) and add 100 ml of  $H_2O$  at 30°. Place rubber stopper in mouth of flask and shake vigorously 1 min. Place flask in constant temp. cabinet or water bath at 30° for 1 hour, shaking ca 10 times every 15 min. Remove flask from cabinet or water bath, add 3 or 4 drops of capryl alcohol, shake 10 times to remove any foam that may be present, and pour suspension into bowl of viscosimeter.

**20.68****DETERMINATION**

After pouring suspension into viscosimeter bowl, make sure that bowl is flush on its supports. Start machine, but before placing bob or disk in place, stir soln with bob 25 times to insure uniform suspension. Place wire of bob in holder and take reading after damping swing of dial by placing a finger on indicator pointer and then gradually touching swinging dial. Make second reading after addition of 1 ml of normal lactic acid, and likewise the third and following readings after addition of 2 ml increments of the normal lactic acid. Do not stop motor between readings. After or during the addition of lactic acid, stir suspension 25 times by up-and-down motion of bob. Suspend bob by the wire and take reading. Determine maximum apparent viscosity of the acidulated flour- $H_2O$  suspension by plotting apparent viscosity readings against volume of acid added. Usually a total of 7 ml of 1 *N* lactic acid is sufficient to give maximum reading, but 2 ml increments should be added continuously until no further increase in apparent viscosity is noted.

**SOYBEAN FLOUR IN UNCOOKED CEREAL PRODUCTS (32)****20.69****Qualitative Test**

Place ca 0.5 g of sample in small test tube containing a strip of red litmus paper partly immersed in 5 ml of 2% soln of urea. Mix, stopper tube, and heat at 40° for 3 hours. If soybean flour is present in more than traces the litmus will be colored blue. (Bromothymol blue may also be used as an indicator, which likewise turns blue if soybean flour is present.)

**RYE, OATS, CORN, BUCKWHEAT, RICE, AND BARLEY AND  
THEIR PRODUCTS EXCEPT CEREAL ADJUNCTS (33)—OFFICIAL****20.70****PREPARATION OF SAMPLE**

Grind sample to pass thru 20-mesh sieve or sieve having circular openings 1/25" (1 mm) in diameter and mix thoroly.

**20.71****MOISTURE.—See 20.2**



20.72

ASH.—See 20.5

20.73

CRUDE FAT OR ETHER EXTRACT.—See 27.25

20.74

CRUDE FIBER.—See 27.30

20.75

PROTEIN (N×6.25).—See 2.24, 2.25, and 2.26

20.76

FAT ACIDITY.—See 20.20 and 20.21

**SOYBEAN FLOUR—TENTATIVE**

20.77

MOISTURE.—See 20.2 or 20.4

20.78

ASH.—See 20.5

20.79

NITROGEN

Proceed as directed in 2.24, 2.25, or 2.26, with the additional option of using sodium alizarin sulfonate or congo red as indicator.

20.80

CRUDE FIBER.—See 27.30.

20.81

CRUDE FAT OR ETHER EXTRACT.—See 20.15

20.82

OIL OR PETROLEUM BENZINE EXTRACT.—See 31.67

**BAKED CEREAL PRODUCTS****BREAD**

20.83

**PREPARATION OF SAMPLE**

(When total solids of original loaf not desired)

(a) *All Types of Bread Not Containing Fruit (33)*—Official.—Cut loaf, or  $\frac{1}{2}$  loaf, of bread into slices 2–3 mm thick. Spread slices on paper and allow to dry in warm room until sufficiently crisp and brittle to grind well in a mill. Grind entire sample to pass 20-mesh sieve, mix well, and keep in air-tight container.

(b) *Raisin Bread—Tentative*.—Proceed as directed in (a) except to comminute by passing twice thru food chopper instead of thru grinder.

20.84

**TOTAL SOLIDS IN ENTIRE LOAF OF BREAD**

(a) *All Types of Bread Not Containing Fruit (33)*—Official.—Accurately weigh loaf of bread immediately upon receipt (A), using scales sensitive to at least 0.2 g. Should accurate weighing be impossible at this time, seal sample in air-tight container and accurately weigh as soon thereafter as is practicable (A). Preserve sample in such manner that no loss of bread solids can occur, whereby loss would be calculated as moisture. Cut bread into slices 2–3 mm thick ( $\frac{1}{2}$  of loaf may be used). Spread slices on paper, allow to dry in warm room (15–20 hours), and when apparently dry, break into fragments. If bread is not entirely crisp and brittle, allow it to dry longer—until it is in equilibrium with moisture of air—in order that no moisture changes may occur during grinding. Quantitatively transfer air-dried bread to scale pan and accurately weigh (B). Grind sample just to pass 20-mesh sieve, mix well, and keep in air-tight container. Determine percentage of total solids (C) of ground sample as directed under 20.3 or 20.4. Calculate total solids of bread from the formula:

$$T.S. = \frac{B \times C}{100} \times 100, \quad \text{or} \quad \frac{B \times C}{.}$$

in which  $A$  = weight of loaf (or  $\frac{1}{2}$  loaf) at time of receipt;  $B$  = weight of the air-dried sliced bread; and  $C$  = percentage of total solids in prepared ground sample.

(b) *Raisin Bread—Tentative.*—Proceed as directed under (a), except to comminute by passing thru food chopper twice instead of thru grinder.

#### 20.85 TOTAL SOLIDS OF AIR-DRIED GROUND SAMPLE (33)—OFFICIAL

Use 2 g of prepared sample, 20.83, and proceed as directed under 20.3 or 20.4.

#### 20.86 FAT AND FAT NUMBER (34)—TENTATIVE

Slice one loaf of bread, place in wire rack, and allow to dry overnight, or until sufficiently dry to grind. Grind bread to ca size of openings in 20-mesh sieve, mix, sample, and transfer 50 g to 600 ml beaker. Add 100 ml of  $H_2O$  and mix. Add 100 ml of  $HCl$ , mix, cover, and heat on steam bath 1 hour, stirring well 6 or 7 times. Cool in cold ( $15^\circ$  or less) water bath, add 50 ml of ice-cold  $H_2O$ , and stir. Add 10 g of Filter Cel, stir, and mix in completely. Prepare 90 mm Büchner funnel as follows:

Place two No. 590 S & S 9 cm filter papers in funnel and apply suction. Mix 10 g of Filter Cel with 50 ml of  $H_2O$  and rapidly pour mixture into funnel. (This should make a smooth even layer of the Filter Cel over the whole filter paper, with no crack or opening.) Filter sample immediately. Rinse out beaker several times with ice-cold  $H_2O$ . Just before filtration is complete, wash down sides of Büchner with ca 100 ml of ice-cold  $H_2O$ . Up to this point do not allow pad to suck dry. Continue with suction until Filter Cel pad seems dry. Transfer this mass, without filter paper, from Büchner to original beaker. Rinse off filter paper and funnel with petroleum benzine and add benzine to beaker containing the dry mass. Break up mass with rod, dry overnight on steam bath and then heat in oven at  $100^\circ$  ca 1 hour to remove all moisture (material must be dry or fat results will be low). Add 25 g of anhydrous  $Na_2SO_4$  and break up any lumps. Prepare large Knorr extraction tube of ca 200 ml capacity (glass tubing 5 cm in diam. with height of 12 cm from shoulder to top of tube). Pack tube with asbestos tamped tightly to form pad ca  $\frac{3}{8}$ " thick. Insert stem of tube into 2-holed rubber stopper in filtering bell jar connected to suction thru 2-way stopcock. Place 500 ml Erlenmeyer flask within bell jar so that stem of tube passes thru neck of flask. To cool beaker and contents add 150 ml of mixed ether and petroleum benzine (1+1) and macerate against sides of beaker with medium sized stiff metal spatula 3-4 min. Decant into extraction tube. Add to beaker 80 ml of the mixed ethers. Work as before 2 min., and decant. Transfer contents of beaker to extraction tube, suck dry, and tamp with flattened stirring rod until all ether is removed. To material in tube add 100 ml of the mixed ethers that have just previously been used to rinse out beaker, mix thoroly with stirring rod a few minutes, allow to stand a minute, then suck dry, and tamp material as before. Make two additional extractions, turning suction on and off carefully to avoid loss of sample in Erlenmeyer flask. Hang thread in flask from top so that it touches the bottom. (Time may be saved by transferring to tall-form 1 liter beaker.) Evaporate on steam bath, completely transfer fat with small amounts of petroleum benzine to tared 150 ml beaker, carefully evaporate benzine on steam bath, dry at  $100^\circ$  to constant weight (ca 30 min.), cool, and weigh. Calculate percentage of total fat on moisture-free basis.

Weigh duplicate samples of 1 g (within  $\pm .03$  g) of fat into 300 ml Florence flasks, add 4 ml of glycerol-soda soln, 31.28(c), and saponify as directed under 31.29. Cool, add few pieces of previously ignited pumice stone, 138 ml of  $CO_2$ -free  $H_2O$ , and 3 ml of  $H_2SO_4$  (1+4), and proceed as directed in 31.29, using same apparatus. Use 0.02  $N$   $NaOH$  for titration and report number of ml per 1 g of fat. Multiply ml of 0.02  $N$   $NaOH$  used by 1.1 and divide by weight of fat taken to obtain "fat number." Run blank determination.

## 20.87

CITRIC ACID (*ss*)—TENTATIVE

To weight of air-dried bread equivalent to 77.7 g of moisture-free bread, in 500 ml volumetric flask, add 400 ml of alcoholic  $\text{H}_2\text{SO}_4$ -phosphotungstic acid soln (25 ml of  $N \text{ H}_2\text{SO}_4$ , 20 ml of 20% phosphotungstic acid soln, and 55 ml of  $\text{H}_2\text{O}$ , made to 500 ml with alcohol). Shake 5 min., make to mark with the  $\text{H}_2\text{SO}_4$ -phosphotungstic acid soln, and allow to stand overnight. Readjust to mark with alcohol, shake 5 min., and filter with suction on paper in 12 cm Büchner funnel. Transfer 325 ml of filtrate to centrifuge bottle, add 30 ml of Pb acetate soln (75 g of the salt +1 ml of acetic acid diluted to 250 ml with  $\text{H}_2\text{O}$ ), shake 5 min., and centrifuge at ca 900 r.p.m. 15 min. Decant supernatant liquid (disregard turbidity), allow to drain, transfer residue with ca 150 ml of  $\text{H}_2\text{O}$  to 250 ml volumetric flask, and thoroly saturate with  $\text{H}_2\text{S}$ . Make to mark with  $\text{H}_2\text{O}$ , shake thoroly, and filter thru large folded filter. Evaporate 200 ml of clear filtrate in 500 ml Erlenmeyer flask over free flame to ca 75 ml. Cool to 45–50°; add 10 ml of  $\text{H}_2\text{SO}_4$  (1+1), 5 ml of KBr soln (15 g in 40 ml of  $\text{H}_2\text{O}$ ), and 15 ml of  $\text{KMnO}_4$  soln (5 g of  $\text{KMnO}_4$  diluted to 100 ml). After ca 2 min., stopper Erlenmeyer, shake vigorously, and allow to stand 3 min. longer. Add 20 ml of  $\text{FeSO}_4$  soln (40 g of the salt +1 ml of  $\text{H}_2\text{SO}_4$  diluted to 100 ml with  $\text{H}_2\text{O}$ ), cool to ca 15°, and shake vigorously until the pentabromacetone has crystallized (lace-like deposit on walls of flask). Place in refrigerator at ca 15° overnight. Avoid temp. of less than 15°, since at lower temp. there is a tendency for the pentabromacetone to freeze on sides of flask. Proceed as directed under 26.37, beginning "Decant supernatant liquid."

## 20.88

ASH (*ss*)—OFFICIAL

Use 3–5 g of prepared sample, 20.83, and proceed as directed under 20.5 or 20.7.

## 20.89

CHLORIDES IN ASH—OFFICIAL.—*See* 20.113

## 20.90

IRON—OFFICIAL, FIRST ACTION.—*See* 20.9

## 20.91

CALCIUM—OFFICIAL, FIRST ACTION.—*See* 20.13

## 20.92

## PROTEIN—OFFICIAL

(Organic and Ammoniacal Nitrogen)

Determine N as directed under 2.24, 2.25, or 2.26, using 2 g of prepared air-dried ground sample, 20.83. Multiply percentage of N by factor 5.7 to obtain percentage of protein.

## 20.93

FAT (ACID HYDROLYSIS METHOD)—OFFICIAL.—*See* 20.16

## 20.94

## CRUDE FIBER—OFFICIAL

(For bread and other baked products not containing fruit.)

Proceed as directed under 27.30.

## 20.95

SUGARS—TENTATIVE.—*See* 27.31 and 27.32

## HYDROGEN-ION CONCENTRATION

## 20.96

*Colorimetric method—Official, First Action.—See* 20.26

## 20.97

*Electrometric method—Official, First Action.—See* 20.27

## BAKED PRODUCTS OTHER THAN BREAD (37)—TENTATIVE

(Not containing fruit)

20.98	SOLIDS.—See 20.84
20.99	ASH.—See 20.5 or 20.7
20.100	PROTEIN.—See 20.92
20.101	FAT.—See 20.16
20.102	CRUDE FIBER.—See 27.30
20.103	SUGARS.—See 27.31 and 27.32

## HYDROGEN-ION CONCENTRATION

20.104	<i>Colorimetric Method—Official.</i> —See 20.26
20.105	<i>Electrometric Method—Official, First Action.</i> —See 20.27

## FIG BARS AND RAISIN-FILLED CRACKERS

## 20.106 MOISTURE—OFFICIAL, FIRST ACTION (39)

Place 25–30 g of prepared sand and short stirring rod in dish of ca 55 mm diam. and 40 mm depth, fitted with cover. Dry thoroly, cover dish, cool in desiccator, and weigh immediately. Remove cover and place 3–5 g of the prepared sample in dish and weigh accurately. Remove dish containing the sand, stirring rod, and weighed charge from balance. Add 5–10 ml of H<sub>2</sub>O and mix with the sand. Heat carefully on water bath, stirring at intervals of 2–3 min. until excess H<sub>2</sub>O is removed and contents of dish are of heavy pasty consistency. Place uncovered dish in vacuum oven and dry for ca 16 hours at 70° under pressure not to exceed 50 mm of Hg. At end of drying period, cover dish, transfer to desiccator, allow to cool to room temp., and weigh immediately.

NOTES: Quartz sand passing thru 40-mesh sieve and retained on 60-mesh sieve, which has been digested with HCl, washed free of acid, and ignited, is recommended. All dishes with fit-over covers are most convenient. The dish can be set in the cover during heating on water bath and during oven-drying period. At termination of drying period the cover can be easily and quickly refitted on dish as it is transferred to the desiccator.

## 20.107 FAT—OFFICIAL, FIRST ACTION (39)

Weigh accurately charge of ca 2 g of well-mixed sample, prepared by grinding twice thru food chopper, and transfer to Mojonnier tube. Add 2 ml of alcohol, warm to 60–70°, and shake gently until charge is thoroly disintegrated and mixed with the alcohol. Add 10 ml of HCl (25+11). Place tube in water bath held at 70–80° and shake at frequent intervals until charge is thoroly digested (40–80 min.).

If weighed charge cannot be transferred to tube before it is digested, or if a Röhrig tube is used, make digestion in 50 ml beaker. Transfer digested mixture to tube as completely as possible by draining from lip of beaker down a small stirring rod. Rinse beaker thoroly with 10 ml of alcohol, transfer to extraction tube, mix thoroly, and cool. Rinse beaker with portions of first 25 ml of ether as the ether is added for first extraction. Repeat rinsing with portions of petroleum benzine (b. p. below 60°) as it is added for first extraction. Rinse thoroly so that all fat is transferred to extraction tube. (At end of digestion period, all particles should be completely disintegrated with exceptions of hard seeds (in fig fillers) and strong fibers. A very small quantity of fat may be retained by such particles after digestion, but for practical purposes in analysis of biscuits and crackers this will be within the tolerance of experimental error.)

When digestion is made in the extraction tube, add 10 ml of alcohol to digested charge and cool. (The level of liquids should be in neck of Mojonnier tube just below pouring-off level or just below drawing-off spigot of Röhrig tube.) Add 25 ml of ether, stopper flask with cork or Neoprene or other synthetic rubber stopper not affected by solvents, and shake thoroly for ca 1 min. Release pressure carefully after tube is shaken so that none of solvent containing fat is lost. Wash adhering solvent and fat from stopper back into extraction tube with few ml of petroleum benzine. (A wash bottle producing fine jet is convenient for this purpose.) Allow mixture to stand few min., then add 25 ml of petroleum benzine (b.p. below 60°), stopper tube tightly, and shake thoroly again for ca 1 min. Release pressure carefully, remove stopper, and again wash adhering solvent and fat back into tube with a few ml of the petroleum benzine. Allow mixture to stand until ether layer is clear (10–20 min.), or centrifuge Mojonnier flask 20 min. at ca 600 r.p.m. Pour off as much as possible of the clear ether-fat soln thru small, fast filter by tilting Mojonnier tube gradually or draw off thru spigot of the Röhrig tube. (A plug of ether-extracted cotton packed just firmly enough in stem of a funnel to allow free passage of ether makes an excellent filter for these extractions.) Catch ether-fat solns from extractions in 250 ml, clean, glass beaker or flask. Re-extract digested charge remaining in tube three times more as directed for first extraction. (Portions of ether may be cut down to 15 or 20 ml for the last 3 extractions.) Wash off mouth of Mojonnier tube or spigot of Röhrig tube each time after draining off ether-fat soln and filter this ether thru funnel into receptacle.

Evaporate combined ethers from extractions from receiving beaker or flask by fanning or suction. After ethers are practically off, heat for ca 10 min. on hot water or steam bath to drive off most of alcohol and H<sub>2</sub>O that has been carried over with ethers. Transfer beaker to 100° air oven, dry for 1 hour, remove, and allow to cool. Redissolve the dried fat in 15–20 ml of a mixture of equal parts of ether and petroleum benzine and filter thru small fat-free filter paper into beaker or flask that has been previously dried at 100°, cooled in desiccator, and weighed.

Wash all traces of fat from first receptacle, filter paper and funnel into the tared beaker or flask with jet of petroleum benzine from wash bottle. Evaporate ethers from tared receptacle by fanning or suction and dry purified fat to constant weight in 100° air oven (1–1½ hours). Cool in desiccator and weigh as soon as room temp. is attained. Make blank determinations on reagents.

**NOTES:** Good quality rubber stoppers thoroly cleaned with alcohol are considered satisfactory for stoppering extraction tubes. Stoppers should be removed from tubes after each shaking period and not allowed to remain in contact with solvents longer than necessary. There is a probability of some solvent action on rubber. Very fine grain cork stoppers that have been washed with alcohol and ether are also considered satisfactory for stoppering extraction tubes, provided leakage of solvents can be prevented during shaking. The fitted ground-glass stopper in the Röhrig tube is most satisfactory.

If trouble is experienced in releasing pressure after shaking extraction tube containing ethers, cool tube slightly by holding it under stream of cold H<sub>2</sub>O before removing stopper.

Al beakers have been found very satisfactory for weighing purified fat as they are light in weight and cool to room temp. rapidly.

## MACARONI, EGG NOODLES, AND SIMILAR PRODUCTS.

### 20.108 COLLECTION AND PREPARATION OF SAMPLE (ss)—OFFICIAL

Select from lot to be analyzed sufficient strips or pieces to assure representative sample, break these into small fragments with hands or in mill, and mix well. Grind 300–500 g in mill until all material just passes thru 20-mesh sieve. Keep ground sample in sealed container to prevent moisture changes.

## TOTAL SOLIDS AND MOISTURE

20.109 *Vacuum Oven Method (39)—Official*

Using prepared sample, 20.108, proceed as directed under 20.3.

20.110 *Air Oven Method—Official*

Using prepared sample, 20.108, proceed as directed under 20.4.

## 20.111 ASH—OFFICIAL

Using 3–5 g of prepared sample, 20.108, proceed as directed under 20.5.

## 20.112 ORIGINAL ASH IN MACARONI PRODUCTS CONTAINING ADDED SALT BUT NOT CONTAINING ADDED EGGS (40)—TENTATIVE

Proceed as directed under 20.5. Dissolve ash in 25 ml of  $\text{HNO}_3$  (1+3), transfer to 150 ml beaker, dilute to 75 ml with  $\text{H}_2\text{O}$ , and boil 15 min., maintaining original volume (this is necessary to convert all phosphate to ortho form). Determine  $\text{P}_2\text{O}_5$  as directed under 2.12.  $\text{P}_2\text{O}_5 \times 2 = \text{NaCl-free ash}$ .

## 20.113 CHLORIDES IN ASH AS SODIUM CHLORIDE (41)—OFFICIAL

Dissolve ash obtained under 20.111 in  $\text{HNO}_3$  (1+9), filter, wash filter paper with hot  $\text{H}_2\text{O}$ , and determine Cl in combined filtrate and washings as directed under 12.42 or 12.44. Calculate Cl to its equivalent of NaCl. (This NaCl value deducted from total ash does not give NaCl-free ash.)

## 20.114 FAT (ACID HYDROLYSIS METHOD) (42)—OFFICIAL

Place 2 g of sample in Röhrig or Mojonnier fat extraction tube, add 2 ml of alcohol to prevent lumping on addition of acid, and shake so as to moisten all particles. Add 10 ml of HCl (25+11), mix well, set tube in water bath held at 70–80°, and shake at frequent intervals 30–40 min. Fill to within 1–2 ml of mark with alcohol and cool. Add 25 ml of ether and shake mixture well. Then add 25 ml of petroleum benzine (b.p. below 60°) and mix well. Let stand until upper liquid is practically clear and proceed as directed under 20.16, beginning "Draw off as much as possible."

## 20.115 CRUDE FIBER—OFFICIAL.—See 27.30

## 20.116 PROTEIN (43)—OFFICIAL

Determine N as directed under 2.24, 2.25, or 2.26, using 1 g of prepared sample, 20.108. Multiply percentage of N by factor 5.7 to obtain percentage of protein.

## 20.117 WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL—TENTATIVE.—See 20.39

## HYDROGEN-ION CONCENTRATION

20.118 *Colorimetric Method—Official.—See 20.26*20.119 *Electrometric Method—Official, First Action.—See 20.27*

## 20.120 LIPOID AND LIPOID PHOSPHORIC ACID (P.O.)—OFFICIAL

Proceed as directed under 20.40 and 20.41.

## UNSATURATED RESIDUE (44)—OFFICIAL

## 20.121 REAGENTS AND APPARATUS

See 23.13 and 23.14. (The conc. KOH soln is not needed.)

## 20.122

## DETERMINATION

Weigh 10 g of sample (ground to pass 20-mesh sieve) into 500 ml Erlenmeyer flask and add with shaking 30 ml of HCl (1+1). Heat on steam bath 30 min., shaking flask occasionally to break up any lumps. While cooling the inclined flask under the tap, add carefully with shaking 30 g of KOH pellets at such a rate that liquid may boil, but not so violently as to cause loss by spurting. While still hot, place flask on steam bath, cover with small watch-glass, and heat for 3 hours, with occasional swirling of mixture to carry down any material adhering to sides. Cool until just warm, add 30 ml of alcohol and 50 ml of H<sub>2</sub>O, and mix well. Add 100 ml of ether, swirl mixture vigorously for 1 min., and transfer to separator, washing flask with 50 ml and 25 ml portions of ether. Wash flask with 50 ml of the dilute KOH soln, pour washings into separator in slow stream while gently swirling the liquid, and continue gentle swirling for 10–15 seconds. Proceed as directed under 23.15, beginning "Allow liquids to separate (ca 10 min.)," but omitting first acid washing. If emulsion forms and does not break to give sharp interface in 10 min., pour 5 ml of alcohol into separator and allow to stand until sharp interface appears.

## 20.123

## STEROLS (AS CHOLESTEROL) (44)—OFFICIAL

Determine the sterols in the unsaponifiable matter as directed in 23.18. However, to the unsaponifiable matter from egg-free products or from any containing less than 0.23% unsaponifiable matter (as is basis), add 10 mg of cholesterol before applying cholesterol method and correct result accordingly. (Added cholesterol should be of highest quality obtainable (m.p. not less than 147°); its purity should be tested by submitting 20 mg to the determination.)

## 20.124

EXTRACTION, SEPARATION, AND IDENTIFICATION OF COLORING  
MATTER\* (46)—OFFICIAL

Place ca 500 g of coarsely ground sample (depending upon quantity of color present) in liter Erlenmeyer flask, add ca 700 ml of 80% alcohol, and shake at intervals 24 hours, or until no more color is imparted to solvent. Place flask with contents in refrigerator overnight to permit dissolved protein matter to precipitate out. Filter, and evaporate filtrate to 100 ml. Add to filtrate ca  $\frac{1}{2}$  volume of 25% NaCl soln and slight excess of NH<sub>4</sub>OH; cool, and transfer to separator. Extract this mixture with equal volume of petroleum benzine, b.p. below 60°, separate lower layer and repeat extractions with additional portions of solvent, until no more color is extracted. Reserve lower layer, if colored, for further treatment; if colorless, discard. Combine petroleum benzine extracts and wash with several small portions of NH<sub>4</sub>OH (1+50) to remove any material mechanically adhering to solvent. This ethereal soln will contain the fats, and also may contain the oil-soluble coal tar dyes, which may be identified by procedure under (1). If colored, immediately acidify alkaline aqueous soln, freed from fat and oil-soluble coal tar dyes, with acetic acid and extract in 25 ml portions with two 50 ml volumes of ether. The solvent, if colored, may contain turmeric, annatto, and trace of saffron. For their identification use procedure (2). If original aqueous soln freed from ether-soluble colors should still be colored, water-soluble dyes may be suspected, in which case the following procedure is recommended: Extract aqueous soln with 50 ml portions of amyl alcohol to remove balance of saffron, as well as common orange dyes (S & J numbers 85, 86, 13) and martius yellow. For their separation proceed as directed under (3). Draw off lower aqueous layer, which, if colored, may contain naphthol yellow S, tartrazine, and sunset yellow. Extract these dyes with amyl alcohol after acidifying soln with HCl to make ca

\* See note, p. 284, for corresponding color numbers

1 *N.* Remove tartrazine from solvent with 0.25 *N* HCl. Sunset yellow will also be removed at this stage with slightly lower acid concentration, and naphthol yellow S from nearly neutral soln. Confirm with wet and spot reactions. Extracted solns are usually very dilute, therefore it is advisable to concentrate by evaporation over steam bath, and if not clear, to add ca 5 ml of 25% NaCl soln to break up slight emulsions by precipitating protein matter. Filter, and test filtrate by dyeing and coupling. This coupling test is carried out as follows: Treat ca 10 ml of filtered soln with excess of Br, destroy excess with saturated hydrazine sulfate soln, and immediately pour into 2 *N* Na<sub>2</sub>CO<sub>3</sub> soln containing a few drops of 1%  $\alpha$ -naphthol soln. In presence of tartrazine or sunset yellow a pink color will be produced. It is advisable to run blank determination on above test for comparison.

(1) Extract original petroleum benzine extract with two or three 10 ml portions of mixture consisting of 1 part of HCl and 5 parts of acetic acid.

In presence of S & J numbers 7 or 16, yellow OB or yellow AB, a pink or red color is obtained. Test small portion of this acid extract with a few drops of SnCl<sub>2</sub>, which in the presence of the above dyes will cause either decolorization or decided fading. Dilute balance of acid extract with H<sub>2</sub>O, make slightly alkaline, and extract color with petroleum benzine. Wash solvent with 2–5 ml portions of H<sub>2</sub>O to remove excess alkali. Test ca 5 ml portion of the petroleum benzine extract with formaldehyde and acetic anhydride as directed under 21.9(a) (1). Evaporate another 5 ml portion of the petroleum benzine extract to dryness in small evaporating dish and observe spot tests with HCl and H<sub>2</sub>SO<sub>4</sub>. Evaporate to dryness balance of petroleum benzine extract in small casserole and dissolve residue in dilute alcohol. Dye some silk strands preferably using slightly alkaline soln. Compare spot tests obtained with Table 1, Chap. 21. If they do not agree, mixture of dyes may be present, which will necessitate separation according to pH. Remaining coloring matters in petroleum benzine extract may be due to natural coloring matter of wheat, or to coloring matter of egg. Coloring principle of egg yolk, lutein, when heated with alcoholic FeCl<sub>3</sub>, will produce a green coloration. However, this test is not specific for lutein, as carotene and xanthophyll give similar reactions.

(2) Wash ether extract with 5 ml portions of H<sub>2</sub>O to remove excess of acid. To remove annatto and traces of saffron, wash successively with 20 ml portions of 5% NaHCO<sub>3</sub> soln. Divide this alkaline soln into two portions. Heat one portion to 60° on steam bath, dye the color on unmordanted cotton, and compare spot tests with a standard. Acidify remaining portion of the alkaline annatto soln with acetic acid and re-extract with ether. Divide ethereal extract into two small casseroles and evaporate to dryness. Dissolve contents of one casserole in 10 ml of NH<sub>4</sub>OH (1 + 9) and impregnate it on strip of cotton or filter paper. An orange yellow to an orange red coloration is obtained, depending upon amount of dye present. Dry filter paper or cotton, add drop of 40% SnCl<sub>2</sub> soln, and again dry. In presence of annatto purple stain is produced. Spot contents of other casserole with H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub>, when a blue and a greenish blue color are obtained. Transfer two portions (ca 10 ml each) of original ether extract, from which annatto has been removed, into test tubes and treat with equal volume of 10% NaOH soln and equal volume of HCl (1 + 1), respectively. In presence of turmeric (curcuma) the alkaline soln will be reddish brown, while the acid soln will be red. Turmeric can further be confirmed by its behavior with H<sub>2</sub>BO<sub>3</sub>. Apply this test as follows: Shake portion of original ether extract with equal volume of 70% alcohol and to this add 1/10 volume of HCl, mix, and divide soln equally into two test tubes. To one tube add a few crystals of H<sub>2</sub>BO<sub>3</sub> and shake. Use other tube as a control. In the presence of turmeric, a red color will be produced after a short time.

(3) To separate and identify saffron and the orange coal tar dyes, dilute the amyl



alcohol extract with two volumes of petroleum benzine and extract the mixed dyes with several 10 ml portions of  $H_2O$ . To a small portion of this aqueous extract add 1/10 volume of acetic acid and add a few mg of dry Na hyposulfite to reduce all the azo dyes. This treatment will not affect the saffron, which can then be re-extracted by amyl alcohol. After washing solvent repeatedly with small portions of  $H_2O$  (to remove decomposition products) evaporate to dryness, and confirm presence of saffron by spot tests. Remainder of color soln after addition of NaCl and acetic acid is re-extracted with amyl alcohol and later fractionated from the solvent for S & J numbers 85, 86, 13, by 5%  $Na_2CO_3$  soln. Martius yellow if present will still remain in the amyl alcohol and petroleum benzine after the removal of the saffron and oranges. In order to prove its presence, evaporate solvent to dryness and dissolve residue with 10 ml of  $NH_4OH$  (1+9). Divide into two test tubes. Add carefully to one a few crystals of Na hyposulfite. The presence of martius yellow will manifest itself by formation of pink soln. To check its presence use other subdivision for dyeing, spotting, etc.

## 20.125

## RAPID METHOD FOR TARTRAZINE (46)—TENTATIVE

Place 800 ml of cold  $H_2O$  and 5 ml of  $NH_4OH$  in liter Erlenmeyer flask and add 200 g of unground sample. Stopper flask and shake at intervals for 3–4 hours, usually sufficient time to disintegrate material. Use glass rod to dislodge material caking on bottom. Centrifuge, and decant the clear supernatant liquid into liter flask, add soln of 50 g of  $MgSO_4 \cdot 7H_2O$  dissolved in 100 ml of  $H_2O$ , 10 ml of 12% silicotungstic acid, and 10 ml of HCl; shake well, and let stand for 1 hour. (This treatment will precipitate almost all protein matter.)

Centrifuge and decant clear soln into liter casserole. (In presence of minute amount of color, soln will appear almost colorless.) Place in liquid 4 pieces of washed wool (ca  $2 \times 2"$ ) and heat on steam bath until coloring matter is absorbed by fiber. (Under ordinary conditions, this will be accomplished by concentration of soln to half original volume.) Remove wool and wash with  $H_2O$  to free from adhering foreign matter. Transfer wool to 125 ml casserole, add ca 25 ml of  $H_2O$  and a few drops of  $NH_4OH$ , and warm over steam bath to remove color from cloth. Discard wool. Evaporate alkaline color soln to dryness. To residue add 25 ml of  $H_2O$  to dissolve the dye, and filter. (Amount of coloring matter recovered is ca 40% of total.) Divide soln into 2 portions; one of 15 ml, the other 10 ml. Place larger portion in small (50 ml) casserole, acidify slightly with HCl, and add small piece of washed wool to absorb the dye. Spot the dyed wool for tartrazine, comparing color with standard of ca same intensity.

To smaller portion of soln, add 2 drops of HCl and an excess of Br water. If a precipitate forms at this stage owing to amount of soluble protein matter present, centrifuge or filter off this precipitate to obtain better results on coupling. Destroy excess of Br with ca 1 ml of saturated aqueous hydrazine sulfate soln and couple immediately with 1 drop of 1% alcoholic  $\alpha$ -naphthol soln in 5 ml of 10% aqueous  $Na_2CO_3$  soln. Formation of orange or pink color indicates presence of tartrazine.

## 20.126

## CAROTENE—TENTATIVE.—See 20.59

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- (33) Ibid., 9, 42 (1926); 15, 72 (1932); 17, 65 (1934); 23, 513, 520, 526 (1940); 25, 645 (1942).
- (34) Ibid., 18, 574 (1935); 19, 86 (1936).
- (35) Ibid., 16, 427 (1933); 19, 86 (1936).
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- (44) Ibid., 24, 75, 143 (1941); 25, 83 (1942).
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## 21. COLORING MATTERS\*

(The numbers in parentheses and brackets following the name of a dye represent in the first instance the number of that dye as listed in "A Systematic Survey of the Organic Colouring Matters," founded on the German of Drs. G. Schultz and P. Julius, 1904, by Arthur G. Green, while the second number is that listed in the Society of Dyers and Colourists' "Colour Index," first edition, January, 1924.)

### 21.1

#### PIGMENTS AND LAKES

Separate the insoluble pigments, ultramarine, lampblack, etc., which are most commonly used as facings, by washing sample with  $H_2O$  and allowing washings to settle. Identify particles of coloring matter by microscopic examination and treat residue or purified coloring matter with chemical reagents.

The pigments occasionally encountered are charcoal or other form of C, ultramarine blue (principally Al, S), prussian blue (principally Fe), and talcum (principally  $SiO_2$ ). Charcoal is indifferent towards the usual chemical reagents and can be burned. Ultramarine blue is stable towards alkalis, but is decomposed by dilute HCl with evolution of  $H_2S$ . Prussian blue is unaffected by dilute HCl, but is decomposed by alkalis. Talcum can be confirmed by the purple coloration obtained by fusing with  $Co(NO_3)_2$  (test for Al).

Lakes are products formed by combining organic coloring matters with metallic salts. They can be prepared from animal or vegetable coloring matters or from coal tar dyes. As a rule they are insoluble in  $H_2O$ , but are readily decomposed by acids with liberation of the coloring matter.

A large proportion of common pigments other than lakes, such as the yellow, brown and red ochres and umbers, are derivatives of heavy metals and contain Fe, Mn, etc. Others, such as the green and blue compounds, including certain green chlorophyll derivatives, may contain Cu. These pigments may be identified by usual tests for respective metals. The analytical properties of insoluble coloring matters are described in various standard works, some of which are listed under the selected references, especially *Farbstofftabellen* by Schultz (1) and *Colour Index*.

### SOLUBLE COLORING MATTERS AND THEIR LAKES

#### SEPARATION BY WOOL DYEING PROCEDURE (2)

### 21.2

#### *Water-Soluble Coal Tar Dyes*

(a) *Wines, fruit juices, distilled liquors, flavoring extracts, vinegars, beers, sirups, non-alcoholic beverages, and similar products.*—Dilute 20–200 ml of sample with 1–3 volumes of  $H_2O$ , neutralize with  $NH_4OH$  (1+9) if necessary, and boil or heat on steam bath with small piece of white woolen cloth (nun's veiling). If mixture contains much alcohol, heat until most of it has been removed; in other cases take out wool after 5–15 min. and rinse with  $H_2O$ . Treat liquid with 3 or 4 drops of HCl for each 100 ml of soln and warm again 10–20 min. with a clean piece of wool. If the wool takes up much coloring matter in either case, the presence of coal tar dyes is indicated.

The basic colors dye the fiber best from neutral or faintly ammoniacal solns and, if present, they will appear on first piece of wool. Acid colors dye from neutral solns, but more readily from those containing free acid. The lichen colors (3) (archil, cudbear, litmus) go readily on wool, however, and many other natural colors, such as

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\* All methods in this chapter are tentative unless otherwise designated.

turmeric, will dye fiber if present in large amount. On the other hand, a few coal tar dyes, especially auramine O and naphthol green B, are quite unstable, and if present in small quantities may give no distinct dyeing. Acid dyes are much more frequently used than basic dyes, and in most cases they may be removed from wool without much decomposition by "stripping" the latter with  $\text{NH}_4\text{OH}$  (1+9) (4). Many natural colors are destroyed by action of the alkali, while others remain unaffected on the fiber.

If behavior with wool in neutral and acid solns indicates presence of acid dyes, rinse colored cloth thoroly with  $\text{H}_2\text{O}$ , cover with  $\text{NH}_4\text{OH}$  (1+9) in a casserole, and boil for a few minutes. Remove cloth and squeeze out adhering liquid. Boil ammoniacal soln to remove excess of  $\text{NH}_3$ , drop in piece of clean wet wool, make distinctly but not strongly acid with  $\text{HCl}$  (1+9), and boil again. If acid coal tar dyes are present, they will usually give a fairly clean, bright dyeing on the second piece of wool. A further purification may be carried out by repeating the stripping and redyeing, tho this procedure is generally accompanied by corresponding loss of dye.

(b) *Candies and similar colored sugar products*.—Dissolve ca 20 g of sample in 100 ml of  $\text{H}_2\text{O}$  and treat soln as directed under (a). When coloring matter is on surface of candy, pour off soln before colorless inner portion has dissolved.

(c) *Jams and jellies*.—Boil mixture of 10–20 g of sample and 100 ml of  $\text{H}_2\text{O}$  with wool in neutral and also in acid soln as directed under (a). For thick jams it is usually better, tho less easy, first to extract the coloring substances by treating product as directed under (d).

(d) *Canned and preserved fruits and vegetables, sausage casings, smoked fish, coffee, spices, etc.*—Macerate 20–200 g of sample with 4–5 times its weight of alcohol, 80% by volume. Allow to stand few hours, pour off solvent as completely as possible, and repeat extraction, using alcohol 70% by volume and containing ca 1% of  $\text{NH}_4\text{OH}$ . (1) Examine separately filtered alcoholic extracts as directed under (a); or, (2) boil ammoniacal soln until practically neutral, complete neutralization with acetic acid, add the neutral 80% alcohol extract, continue evaporation until most of alcohol is removed, and boil a small portion with wool as directed under (a).

(e) *Cocoa and chocolate products*.—Treat cocoa as directed under (d). Alcoholic extract will contain large quantities of natural coloring matters, and several dyeings and strippings may be necessary to remove these in order to show presence of coal tar dyes.

Chocolate may be treated similarly, but following procedure is preferable: Wash 20–200 g of well-divided sample with gasoline (b. p. 90–120°) on filter until most of fat has been removed; if gasoline is colored, reserve for examination of oil-soluble dyes as directed under 21.3. Remove most of adherent solvent from residue by evaporation or pressure between layers of absorbent paper and digest with alcohol as directed under (d).

(f) *Cereal products (macaroni or other alimentary products)*.—Use 500 g of coarsely ground sample and proceed as directed under 20.124.

## 21.3

### *Oil-Soluble Coal Tar Dyes (5)*

Prepare alcoholic soln of dye by applying one of following methods to oil or fat<sup>1</sup> obtained by extraction with ether or gasoline (b. p. 90–120°) if nature of substance requires it:

(a) Shake oil or melted fat with equal volume of alcohol, 90% by volume, and wash alcoholic extract with several portions of gasoline to free coloring matter from foreign fats. The alcohol, after separation, will contain aniline yellow, butter yellow, aminoazotoluene, auramine, sudanis, yellow OB, yellow AB, etc., if present.

(b) Saponify 20–200 g of the oil or fat with 0.5 *N* alcoholic KOH, remove most of alcohol on steam bath, and extract the soap with gasoline. Remove dyes from solvent with 10 ml portions of mixture containing 1 part of HCl and 5 parts of acetic acid. Most of common dyes are removed by this treatment, tho digestion with strong alkali may cause some decomposition and make extraction rather troublesome.

(c) Proceed as directed under 31.57. The dyeing test is sometimes unsatisfactory, and in all cases small portions of the alcoholic soln should be tested by treating with equal volumes of HCl and  $\text{SnCl}_2$  soln. The common oil-soluble coal tar dyes are rendered more red or blue by the acid and are decolorized by the reducing agent. Most natural coloring matters become slightly paler with the acid and are little changed by the  $\text{SnCl}_2$  soln.

#### SEPARATION BY IMMISCIBLE SOLVENTS PROCEDURE (6)

##### 21.4

##### *Coal Tar Dyes in General*

The use of immiscible solvents for the separation of mixtures of coloring matters generally requires a systematic fractionation since many dyes do not differ very greatly in their solubilities in various solvents.

##### 21.5

##### PREPARATION OF SOLUTION

(a) *Water-soluble colors*.—Proceed as directed under 21.2, omitting fixation of the color on wool, and obtain an aqueous soln as free as practicable from suspended matter, alcohol, acids, alkalies, and salts. Liquids require no preparation except removal of any alcohol that may be present.

(b) *Water-insoluble lakes*.—If sample is in solid form, treat well-divided material with sufficient  $\text{H}_2\text{O}$  to form a paste.

(c) *Oil-soluble dyes*.—Proceed as directed under 21.3, preferably 21.3(a) or 21.3(c).

##### 21.6

##### *Basic Dyes*

Most basic dyes may be separated from mixtures by making alkaline with 10% NaOH soln and shaking with ether (7). Use prepared soln, 21.5, for this purpose. Separate ether layer, which may or may not be colored; wash twice with a few ml of  $\text{H}_2\text{O}$  to remove excess of alkali; and shake with acetic acid (1+18), which will take up any dye present and form a colored soln. Altho this treatment may, to some extent, alter the common basic colors, it can be used for detection of methyl violet B (451) [680], magenta (448) [677], bismarck brown (197) [331], malachite green (427) [657], and rhodamine B (504) [749]. With care auramine (425) [655] also may be separated in this way, tho it is quickly decomposed on standing in alkaline soln.

##### 21.7

##### *Acid Dyes*

The following short procedure is often convenient for examination of mixtures of acid dyes: Make prepared sample, 21.5, strongly acid by adding  $\frac{1}{2}$  its volume of HCl, and shake with amyl alcohol. Separate amyl alcohol soln and wash by shaking with successive portions of  $\frac{1}{2}$  its volume of  $\text{H}_2\text{O}$ , reserving portions in separate test tubes or beakers. Because of varying acid content of the amyl alcohol these washings will show regular decrease in acidity, and the coloring matters will appear in maximum quantity in the different fractions according to their respective solubilities. Ponceau 6R (108) [186] is washed out chiefly while acidity is still high, approximately normal. Amaranth (107) [184], brilliant scarlet (106) [185], tartrazine (94) [640], sunset yellow FCF, orange G (14) [27], and soluble blue (480) [707] appear when washings

have an acidity of ca 0.25 *N*, and palatine scarlet (53) [77], ponceau 2R (55) [79] and 3R (56) [80], ponceau SX, naphthol yellow S (4) [10], cochineal (706) [1239], crystal ponceau (64) [89], and azorubine A (103) [179] between  $\frac{1}{16}$  *N* and 1/256 *N*. When practically all acid is removed, orange I (85) [150], orange II (86) [151], and croceine orange (13) [26], begin to wash out, and less readily, orange IV (88) [143] and metanil yellow (95) [138]. Finally the unsulfonated coloring matters, such as erythrosine G (516) [772], erythrosine B (517) [773], and the rose bengals (520) [777] and (523) [779] are removed very slowly by H<sub>2</sub>O or not at all unless solvent is diluted with gasoline and dyes are removed with H<sub>2</sub>O containing a few drops of NH<sub>4</sub>OH. Acid yellow (8) [16] and brilliant yellow S (89) [144] are not very uniform in composition. They are partially taken up by amyl alcohol from acid soln and appear chiefly in the first washings. Indigotine (692) [1180] behaves somewhat similarly.

When it appears probable that only the coal tar dyes listed in the regulations for the enforcement of the Federal Food and Drugs Act (8) for use in food products are present, the following abridged procedure may be conveniently used for their separation:

#### PERMITTED COAL TAR FOOD COLORS (9)

(Amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, light green SF yellowish, fast green FCF, guinea green B, brilliant blue FCF, indigotine, naphthol yellow S, sunset yellow FCF, tartrazine, yellow AB, yellow OB, orange SS and oil red XO.)

#### 21.8

##### PREPARATION OF SOLUTION

(a) *For foodstuffs containing oil-soluble dyes.*—Proceed as directed under 21.3(a), evaporate the 90% alcoholic extract to dryness in casserole, treat residue with 40 ml of low-boiling gasoline (b. p. 90–120°), and shake gasoline soln with 2 or 3 portions of 5 ml each of 2–4% NaOH soln (to remove annatto, turmeric, etc., if present). The gasoline soln will contain the yellow OB, yellow AB, orange SS, and oil red XO.

(b) *For foodstuffs that contain no oil-soluble dyes or from which these dyes have been removed.*—Proceed as directed under 21.2, omitting fixation of color on wool, and obtain an aqueous soln as free as possible from suspended matter, alcohol, acids, alkalis, and salts. The dye soln should be preferably between 0.01 and 0.05%. The soln obtained in the examination of colored food products rarely requires further dilution, but with commercial food colors care must be taken that the concentration is not too great.

#### 21.9

##### SEPARATION

(a) *Yellow AB and yellow OB.*—Extract gasoline soln of these dyes, 21.8(a), 3 times with  $\frac{1}{2}$  its volume of 13 *N* H<sub>2</sub>SO<sub>4</sub>. Shake each acid extract successively with 2 portions (equal volumes) of low-boiling gasoline, using same 2 portions of gasoline for each acid portion. Extract each of 2 latter gasoline portions with 20 ml of 13 *N* H<sub>2</sub>SO<sub>4</sub>, using same acid portion successively for both gasoline portions. Finally extract second of these gasoline portions with another 20 ml portion of 13 *N* H<sub>2</sub>SO<sub>4</sub>. (Original gasoline soln has now been shaken with acid 3 times, the next gasoline portion 4 times, and the third 5 times.) Combine acid extracts, dilute with H<sub>2</sub>O, re-extract with low-boiling gasoline, and evaporate solvent. Yellow AB will be found in practically pure state. Combine gasoline solns (original and subsequent solns left after acid washings), wash with small portions of H<sub>2</sub>O to remove excess of acid, and evaporate solvent. Yellow OB will remain as residue. (This method is not absolutely quantitative, but it is sufficiently accurate to make a separation of

either of the dyes with comparatively little contamination from the other.) Following color tests may be applied to separated dyes to confirm their identity:

(1) Shake 5 ml of neutral gasoline soln of dye in test tube with 5 ml of mixture of 1 part of 40% HCHO soln and 4 parts of acetic anhydride. Both coloring matters are extracted by the acetic anhydride, yellow AB giving in few seconds a red colored soln, and yellow OB, under the same conditions, giving orange colored soln.

(2) To 1 ml of alcoholic soln of dye (0.005–0.01%), add 0.1 ml of HCHO, 0.1 ml of H<sub>2</sub>SO<sub>4</sub>, and finally 8.0 ml of H<sub>2</sub>O. Yellow AB gives a red color, unaltered by addition of excess NH<sub>4</sub>OH and somewhat intensified by further addition of excess acetic acid. Yellow OB gives a yellow or orange color.

(3) To 1 ml of alcoholic soln of dye (0.005–0.01%), add 0.1 ml of Cu-pyridine soln (5 g of CuSO<sub>4</sub>·5H<sub>2</sub>O and 10 ml of pyridine made to 100 ml with H<sub>2</sub>O) and 8.0 ml of H<sub>2</sub>O. Yellow AB gives a pink color, becoming purple on addition of excess NH<sub>4</sub>OH. Yellow OB gives a colorless or bluish soln.

(b) *Amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, light green SF yellowish, fast green FCF, guinea green B, brilliant blue FCF, indigotine, naphthol yellow S, sunset yellow FCF, and tartrazine.*—To soln obtained under 21.8(b), add sufficient 25% NaCl soln to make concentration ca 10%, and 1 part acetic acid to every 7 parts of soln. Extract with 3–50 ml portions of amyl alcohol. Draw off the lower layer and reserve for further treatment. Wash the amyl alcohol extract in rotation with 25 ml portions of 5% NaCl soln until washings are colorless or nearly so. Add washings to original aqueous soln. Dilute amyl alcohol extract with equal volume of gasoline and wash with 25 ml portions of H<sub>2</sub>O until all color is extracted. Coloring matters obtained are orange I and guinea green B. For their separation see (1) below. Treat the amyl alcohol-gasoline soln with 10 ml portions of 0.1 N NaOH or with 10 ml portions of NH<sub>4</sub>OH (1+9), which will remove erythrosine. Acidify original soln and washings (from which the 3 named dyes were removed) with HCl (1 volume acid to 40 volumes of soln) and extract in 50 ml volumes with three 50 ml portions of amyl alcohol. Reserve lower aqueous layer for further treatment. Wash the amyl alcohol extract with 25 ml portions of 0.25 N HCl until washings are colorless or nearly so. Combine washings with aqueous soln above. Extract the amyl alcohol with several 25 ml portions of H<sub>2</sub>O until all color is extracted. Coloring matters obtained are ponceau 3R, ponceau SX, and naphthol yellow S. For their separation see (2). Treat original soln and washings (from which the 6 named dyes were removed) in 50 ml volume with 3–50 ml portions of  $\alpha$ -dichlorhydrin. Reserve upper aqueous layer for further treatment. Wash the dichlorhydrin extract in rotation with several 20 ml portions of 25% NaCl soln. Combine washings with aqueous soln above. Dilute dichlorhydrin extract with 2 volumes of CCl<sub>4</sub> and extract with several 25 ml portions of H<sub>2</sub>O until all color is extracted. Coloring matters obtained are light green SF yellowish, fast green FCF, and brilliant blue FCF. For their separation see (3). Further acidify original soln and washings (from which the 9 named dyes were removed) with HCl (1 vol. acid to 40 vol. soln) and extract in 50 ml volumes with three 50 ml portions of amyl alcohol. (If color intensity of soln was not too strong, all coloring matter should have been extracted by solvent.) Discard lower colorless or nearly colorless layer and wash out dyes from amyl alcohol extract in rotation with several 25 ml portions of H<sub>2</sub>O, until all color is extracted. Coloring matters obtained are indigotine, amaranth, tartrazine, and sunset yellow FCF. For their separation see (4).

(1) *Orange I and guinea green B.*—Extract combined colors with two 20 ml portions of  $\alpha$ -dichlorhydrin. Discard colorless upper aqueous layer, dilute solvent with 2 volumes of CCl<sub>4</sub>, and extract orange I in rotation with several 10 ml portions of H<sub>2</sub>O, and guinea green B with several 10 ml portions of 25% alcohol.

(2) *Ponceau 3R, ponceau SX, and naphthol yellow S*.—Acidify combined colors with HCl (1 part acid to 10 parts of soln) and extract naphthol yellow S with two 20 ml portions of washed ethyl acetate or amyl acetate. (Ponceau 3R and ponceau SX are not extracted appreciably and remain in aqueous layer.) Wash solvent with 5 ml portions of normal HCl to remove traces of the ponceaus. Remove naphthol yellow S from combined ethyl acetate or amyl acetate extracts with 5 ml portions of  $\text{NH}_4\text{OH}$  (1+9). Extract remaining ponceau soln with 20 ml portions of amyl alcohol and wash out excess of acid twice with a few ml portions of  $\text{H}_2\text{O}$ . Dilute amyl alcohol with equal volume of gasoline, and remove color with small volumes of  $\text{H}_2\text{O}$ . Treat 10 ml of this soln with 1 ml of HCl, 2 ml of strong Br water, and lastly 3 ml of saturated hydrazine sulfate soln; immediately pour into test tube containing 10 ml of 2 *N*  $\text{Na}_2\text{CO}_3$  and 2 drops of 1% alcoholic  $\alpha$ -naphthol. (Light orange soln indicates ponceau 3R, deep brownish red soln indicates ponceau SX.) Add to soln 5 ml of ether, mix well, and draw off lower aqueous layer which, if colored, contains ponceau SX. To ethereal extract add equal volume of HCl; formation of purplish soln confirms presence of ponceau 3R.

(3) *Light green SF yellowish, fast green FCF, and brilliant blue FCF*.—Treat combined colors with equal volume of 2 *N*  $\text{Na}_2\text{CO}_3$  soln and extract in 25 ml volumes with two 50 ml portions of normal butyl alcohol. Draw off lower aqueous layer containing the fast green FCF and wash out last traces from solvent with 25 ml portions of 2 *N*  $\text{Na}_2\text{CO}_3$ . Reserve washings and add to aqueous soln for confirmatory tests. Light green SF yellowish is colorless in the solvent while brilliant blue FCF imparts a bluish green color. To prove presence of light green SF yellowish in presence of brilliant blue FCF proceed as follows: Dilute solvent with equal volume of gasoline and remove color with small portions of  $\text{H}_2\text{O}$ . Treat 20 ml of soln with 4 ml of 10% NaOH soln and boil 5 min. Brilliant blue FCF is changed to a red phase, while light green SF yellowish is changed to a yellow. Acidify with 10 ml of acetic acid, which changes brilliant blue FCF to a violet and light green SF yellowish to a green. Treat with ca 3 g of Zn dust and heat until soln is decolorized. Filter, make slightly alkaline with  $\text{NH}_4\text{OH}$  and then acid with acetic acid, and bring to boil. In presence of light green SF yellowish a deep green soln is formed while brilliant blue FCF remains colorless.

(4) *Indigotine, amaranth, tartrazine, and sunset yellow FCF*.—To separate indigotine heat a small portion of soln, which should be neutral or faintly acid, to boiling, and add a few crystals of  $\text{Na}_2\text{S}_2\text{O}_4$  until all dyes are reduced. On adding a few drops of acetic acid and shaking with air the indigotine is quickly restored, while amaranth, tartrazine, and sunset yellow FCF are destroyed. If a positive test for indigotine is obtained, add to remainder of mixed dye soln several decigrams of urca, heat, and while mixture is boiling add 1 or 2 drops of 10%  $\text{NaNO}_2$  soln. Indigotine is converted to the pale yellow isatine sulfonate, while amaranth, tartrazine, and sunset yellow FCF are but little affected. Acidify resultant mixture with  $\text{H}_2\text{SO}_4$  (1+4), using 1 part of dilute acid to 10 parts of soln. Extract in 25 ml portions with three 50 ml portions of normal butyl alcohol. Draw off lower layer and pass successively thru all separators. Reserve aqueous layer if colored; if not colored, discard. Prepare following soln: 13.5 ml of  $\text{H}_2\text{SO}_4$ , 100 g of anhydrous  $\text{Na}_2\text{SO}_4$ , and sufficient  $\text{H}_2\text{O}$  to make 1 liter. Extract the butyl alcohol successively with 25 ml portions of this soln until washings are colorless. Reserve them for amaranth and tartrazine. Dilute the butyl alcohol with equal volume of gasoline and remove sunset yellow FCF with  $\text{H}_2\text{O}$ . Confirm with dyeing tests and wet reactions.

Acidify reserved soln with HCl (1 vol. acid to 20 of soln) and extract with two 30 ml portions of amyl alcohol. (This will extract both amaranth and tartrazine while the isatine compound, being less readily extracted, remains in lower layer and



TABLE 1.—Color reactions produced on dyed fibers by various reagents

COLORING MATTER	C. I. NO.	S. & J. NO.	STRONG HYDROCHLORIC ACID	CONCENTRATED SULFURIC ACID	10% SODIUM HYDROXIDE SOLUTION	AMMONIUM HYDROXIDE
Rhodamine B	749	504	Orange	Yellow	Blue	Blue
Rose Bengal	779	523	Almost decolorized	Orange	No change	No change
Orchil	1842	710	Red	Reddish brown	Violet	Violet
Magenta	677	448	Yellowish brown	Yellowish brown	Decolorized	Paler
Acid Magenta	692	462	Almost decolorized	Yellow	Decolorized	Decolorized
Palatine Red	85	62	Darker	Blue	Dull brown	Little change
Bordeaux B	88	65	Violet	Blue	Brick red	Little change
Amaranth	184	107	Slightly darker	Violet to brownish	Dull brownish to orange red	Little change
Azorubine A	179	103	Little change	Violet	Red	Red
Erythrosine	773	517	Orange-yellow	Orange-yellow	No change	No change
Ponceau 6RB	286	169	Blue	Blue	Dull violet-red	Little change
Ponceau 6R	186	108	Violet-red	Violet	Brown	Orange-red
Crystal Ponceau	89	64	Red	Violet	Dull brown	Little change
Ponceau 3R	80	56	Little change	Little change	Dull orange	Little change
Ponceau SX	...	...	Deeper red	Deeper red	Orange yellow	Orange yellow
Sudan III*	248	143	Violet, then brown	Green	Violet-red	Little change
Safranin	841	584	Greenish blue	Green	Red	Red
Brilliant Scarlet	185	106	Red	Violet-red	Yellowish brown	Orange-red
Ponceau 2R	79	55	Little change	Little change	Brownish yellow	No change
Palatine Scarlet	77	53	Darker	Violet-red	Brownish yellow	No change
Erythrosine G	772	516	Yellow-orange	Yellow-orange	No change	No change
Sudan II*	73	49	Red	Violet-red	Little change	No change
Sudan I*	24	11	Orange-red	Red	Redder	No change
Cochineal	1239	706	Little change	Little change	Violet-red	Violet-red
Bismarck Brown R	331	197	Redder, darker	Browner	Yellow	Yellow
Bismarck Brown R	332	201	Redder, darker	Browner	Yellow	Yellow
Orange I	150	85	Violet	Violet	Red, dark	Red, dark
Orange II	151	86	Red	Red	Dull red	No change
Croceine Orange	26	13	Orange-red	Orange	Slightly darker	No change
Orange G	27	14	Little change	Orange	Dull, brownish red	No change
Orthotoluenearazobeta-naphthylamine* (Yellow OB)	61	...	Red	Violet	Little change	No change

		...	Red	Violet	Little change	No change
Benzeneazobeta-naphthylamine* (Yellow AB)	22	...	Cherry red	Cherry red	Little change	No change
Orange SS*	...	...	Cherry red	Cherry red	Slightly yellow	No change
Oil red XO*	...	...	Orange-yellow	Brownish yellow	Slightly yellow	No change
Sudan G*	23	10	Orange-yellow	Orange-yellow	Orange-yellow	No change
Butter Yellow*	19	16	Violet-red	Orange-yellow	No change	No change
Aniline Yellow*	15	7	Violet-red	Orange-yellow	Little change	No change
Aminoazorthotoluene*	17	...	Dull orange	Orange-yellow	Little change	No change
Fluorescein	766	510	Little change	Little change	Green fluorescent	Green fluorescent
Metanil Yellow	133	95	Violet-red	Violet	No change	No change
Azoflavine	145	92	Violet-red	Violet-red	Little change	Little change
Acid Yellow	16	8	Red	Orange	Dull brown	No change
Brilliant Yellow S	144	89	Violet-red	Violet-red	Little change	No change
Tartrazine	640	94	Slightly darker	Slightly darker	Little change	Little change
Sunset Yellow FCF	...	...	Slightly redder	Slightly redder	Browner	Little change
Naphthol Yellow S	10	4	Almost decolorized	Very pale, dull brown	No change	No change
Auramine	655	425	Decolorized	Almost decolorized	Decolorized	Paler
Turmeric	1238	707	Red	Reddish brown	Orange	Orange
Quinoline Yellow	801	667	Slightly darker	Brownish yellow	Slightly paler	Little change
Naphthol Green B	5	398	Yellowish	Brownish yellow	No change	No change
Guinea Green B	666	433	Pale orange-yellow	Yellowish brown	Decolorized	Decolorized
Light Green SF Yellowish	670	435	Pale orange-yellow	Yellowish brown	Decolorized	Decolorized
Fast Green FCF	...	...	Orange	Green to brown	Blue	Blue
Brilliant Blue FCF	...	...	Yellow	Yellow	No change	No change
Night Green 2B	667	438	Pale orange-yellow	Yellowish brown	Decolorized	Paler
Malachite Green	657	427	Almost decolorized	Almost decolorized	Decolorized	Decolorized
Erioglaucine A	671	436	Yellow	Pale, dull yellow or brown	Slightly darker	Little change
Patent Blue A	712	442	Pale orange-yellow	Green to brown	Little change	Little change
Soluble Blue	707	480	Paler	Brown	Pale reddish	Almost decolorized
Indigotine	1180	692	Slightly darker	Darker	Greenish yellow	Greenish blue
Formyl Violet	698	468	Pale orange-yellow	Pale, dull orange	Decolorized	Decolorized
Methyl Violet	680	451	Yellowish	Yellowish	Decolorized	Almost decolorized
Nigrosine, soluble	865	602	Dull bluish	Dull greenish	Brownish red, paler	Pale reddish

\* Oil-soluble.

is discarded.) Remove coloring matter with several 10 ml portions of  $H_2O$ . To a portion of the soln add 5 drops of  $NH_4OH$  and a few crystals of  $Na_2S_2O_4$ . (This treatment will destroy amaranth completely, leaving tartrazine practically unaltered.) Add excess of  $HCl$  and speedily extract dye with small volume of amyl alcohol, from which soln tartrazine can be removed with 0.25  $N$   $HCl$ . Treat another 10 ml portion of the neutral dye soln in test tube with 2 ml of 20%  $NH_4Cl$  soln and 1 ml of 25%  $KCN$  soln and heat in boiling water bath 5 min. Cool rapidly, acidify with 2 ml of  $HCl$ , and extract with 10 ml of amyl alcohol (caution). Draw off lower layer and discard. Remove tartrazine with 5 ml portions of 0.25  $N$   $HCl$ ; amaranth is converted to a lower sulfonated dye, and is not removed at this acid concentration. Dilute solvent with equal volume of gasoline and extract dye with small volumes of  $H_2O$  (amaranth is modified to brownish red dye).

## 21.10

## IDENTIFICATION (10)

The most widely used tests for identification of coal tar dyes refer to changes produced with acids and alkalis. Other tests, based upon behavior with reducing agents, followed perhaps by treatment with oxidants or by separation and identification of the reduction products (11), and tests based upon oxidation of the dye and treatment of the oxidation products (12), are generally applicable. Spectroscopic methods are also used (13).

## 21.11

*I. By Color Changes Produced with Acids and Alkalies*

Transfer separated coloring matter to wool (or to silk in case of oil-soluble dyes) by boiling as directed under 21.2(a) or 21.3. (Care should be taken that final dyeing is made in soln fairly free from foreign matter such as sugar or aromatic substances, which, adhering to the fiber, may modify reaction. In most cases, quantity of color available is small and should not be used to dye too large a piece of wool, or silk.) Rinse dyed fiber thoroly in running  $H_2O$ , dry, cut into small pieces, and place separately in depressions of white porcelain spot plate. Moisten pieces with  $HCl$ ,  $H_2SO_4$ , 10%  $NaOH$  soln, and  $NH_4OH$ . (For many coloring matters the hue upon treatment with acids or alkalis varies markedly with concentration of reagents and quantity of dye present; therefore unknown dye should be compared with dyeings of known colors, of approximately same dye concentration as shown by their appearance.)

Table 1 shows color changes produced on wool dyed with 0.1–0.5% solns of the respective coloring matters. Included also are the reactions of the oil-soluble colors, but these refer to dyeing on silk. Dyes are arranged approximately according to hue. Brown is classed with orange; black (gray), with violet.

## 21.12

*II. By Special Tests*

(a) *Oil-soluble dyes (yellow AB, yellow OB, orange SS, and oil red XO).*—Alcoholic solns of these dyes become red on treatment with  $HCl$ ; are unaffected by alkalis; are reduced by  $SnCl_2$ ,  $TiCl_3$ , and  $Na_2S_2O_4$ ; and color is not restored to reduced solns on addition of  $FeCl_3$  or  $K$  persulfate.

(b) *Water-soluble dyes (amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, light green SF yellowish, fast green FCF, guinea green B, brilliant blue FCF, indigotine, naphthol yellow S, sunset yellow FCF, and tartrazine).*—Treatment of these dyes in acid soln with  $SnCl_2$ ,  $TiCl_3$ ,  $Zn$  dust, or  $Na_2S_2O_4$  decolorizes indigotine, amaranth, ponceau 3R, ponceau SX, orange I, sunset yellow FCF, and tartrazine. With in-

digotine, color returns on shaking with air, but more readily on warming or on addition of  $\text{FeCl}_3$  or K persulfate. Excess of reducing agents must be avoided. With last 6 named dyes, color is not restored. Dilute solns of light green SF yellowish, guinea green B, fast green FCF, brilliant blue FCF, naphthol yellow S, and erythrosine become yellow or colorless with acid, so that effects of acid-reducing agents are not so readily apparent. Neutral solns of naphthol yellow S are first changed to pink and later decolorized by  $\text{Na}_2\text{S}_2\text{O}_4$  and other reducing agents, the color not returning with air or oxidants. Erythrosine, light green SF yellowish, fast green FCF, brilliant blue FCF, and guinea green B become paler with  $\text{Na}_2\text{S}_2\text{O}_4$ , color being partially restored upon addition of K persulfate.

In hot solns containing an excess of Na tartrate, water-soluble dyes are readily decolorized by  $\text{TiCl}_3$  (14). In the case of indigotine, if the reducing agent has been added carefully and an excess avoided, the blue color readily returns on shaking with air. With erythrosine, light green SF yellowish, fast green FCF, brilliant blue FCF, and guinea green B, color is scarcely restored by air, but on cooling and adding K persulfate it returns imperfectly. Reduction products of the other dyes do not give colored solns again on oxidation, if a slight yellowish or brownish tint that may sometimes appear is disregarded.

(1) *Light green SF yellowish, fast green FCF, brilliant blue FCF, and guinea green B* belong to the triphenyl-methane type of dyes. Solns of light green SF yellowish and guinea green B behave similarly with acids, alkalies, and reducing agents, producing a yellow to a greenish yellow with mineral acids, and an almost colorless soln with alkalies as well as with reducing agents. On the other hand, while the reactions of fast green FCF and brilliant blue FCF are similar with acids and reducing agents, they differ in respect to their behavior to alkalies. While light green SF yellowish is decolorized by the addition of  $\text{NH}_4\text{OH}$  or 10%  $\text{NaOH}$ , fast green FCF produces a deep blue soln by similar treatment, which is not altered even on boiling; brilliant blue, on the other hand, is not affected by  $\text{NH}_4\text{OH}$  or fixed alkalies in the cold, but is changed to a reddish purple soln upon boiling with 10%  $\text{NaOH}$ . The easy solubility of these 4 colors in  $\alpha$ -dichlorhydrin differentiates them from all other permitted dyes. To separate guinea green B from light green SF yellowish, fast green FCF and brilliant blue FCF, proceed as follows:

*Light green SF yellowish and guinea green B.*—Prepare soln of 250 g of NaCl, 27 g of crystallized Na acetate, and 24 ml of acetic acid in  $\text{H}_2\text{O}$ , and dilute to 1 liter.

To separate and differentiate the 2 green coloring matters add to every 20 ml of dye soln 1 ml of  $\text{HCl}$ , and extract with equal volume of amyl alcohol. Draw off lower layer and remove light green SF yellowish by washing remaining amyl alcohol portion with equal volumes of the NaCl-Na acetate soln until no more color is extracted. Dilute the amyl alcohol with an equal volume of gasoline and remove the guinea green B with  $\text{H}_2\text{O}$ .

*Light green SF yellowish, fast green FCF, and brilliant blue FCF.*—To separate and differentiate proceed as directed under 21.9(b)(3).

(2) *Indigotine* is extracted in small proportions from slightly acid solns by shaking with  $\alpha$ -dichlorhydrin, from which it may be removed with small portions of 25% NaCl soln. Most other common bluish dyes are triphenyl-methane derivatives and are relatively more soluble in solvent than in aqueous layer. Indigo is readily destroyed by boiling with a very small amount of a fixed alkali soln, by which treatment it may be easily eliminated from other coloring matters.

(3) *Ponceau 3R* gives in neutral or faintly acid solns a bluish red, flocculent precipitate with  $\text{BaCl}_2$  or Ba acetate, practically all the dye being removed from soln. Some of soln obtained in separation, 21.9(b), may be used in this test, free  $\text{HCl}$  first

being neutralized with Na acetate; or better, soln may be evaporated to dryness on steam bath to remove the acid and residue taken up with a little  $H_2O$ . A brick red precipitate will be formed on standing, when a neutral soln of the dye is treated with a 20% neutral Pb acetate soln. Soln should contain 0.005% or more of the dye.

(4) *Naphthol yellow S*, in solns containing an excess of  $NH_4OH$  or  $Na_2CO_3$ , becomes intensely rose-red on addition of  $Na_2S_2O_4$ , color gradually fading again as complete reduction takes place. Red coloration is also produced if an aqueous soln of the dye is treated with a few drops of 40%  $SnCl_2$  and an excess of 20%  $KOH$  soln is added.

(5) *Tartrazine* is characterized by its comparative inactivity toward acids and alkalis, the soln of the dye being hardly altered by these reagents. An alkaline soln of the dye is reduced with  $Na_2S_2O_4$  only with difficulty. A concentrated neutral or slightly acid soln of the dye, when reduced with  $SnCl_2$  soln or Zn dust and made slightly alkaline and filtered, will develop a purple coloration on standing.

(6) *Orange I* can readily be recognized by its behavior toward reagents. With a large excess of  $HCl$  it produces a purplish-red; with alkali in large excess it produces a bright red soln.

(7) *Erythrosine* differs from most of the common dyes in that it contains I. To test for I, acidify soln with  $H_2SO_4$ , shake with ether, separate ether soln of color, and evaporate to dryness in Pt dish after addition of a few drops of  $Na_2CO_3$  soln or sufficient to form the deep red Na salt. Hold dish containing residue in the Bunsen flame until organic matter is destroyed, take up residue with  $H_2O$ , acidify with  $H_2SO_4$ , and test for I in one of the usual ways, such as with  $Cl$  water and  $CS_2$ , or  $CCl_4$ , or with starch paste and an oxidizing agent. It is useless to test for I with very small quantities of dye, but in most cases sufficient coloring matter can be separated from the food product to give satisfactory results.

## 21.13

### NATURAL COLORING MATTERS

As a class the natural coloring matters show much less tendency to dye animal fiber than do the common synthetic colors. In many cases the crude products used contain a number of colored substances, and a complete separation is not practicable. As dilute solns of most of the natural coloring matters are sensitive to alkalis, and some are sensitive to acids, such reagents must be used with care. Relatively few good tests are known for the common natural colors. Some of their most useful analytical properties (15) are given in Table 2.

The properties of pure preparations of the various natural coloring matters are described, for the most part, by Rupe (16), and by Perkin and Everest (17), reference being made in these works to the original literature. Properties of the chlorophylls and carotenoids are given by Willstätter and Stoll (18), those of the coloring matters of the cornflower, rose, pelargona flower, larkspur, cranberry, whortleberry, purple grape, sloe, cherry, plum, radish, and red beet by Willstätter and coworkers (19).

## 21.14

### SEPARATION

(a) *By extraction with ether from neutral solns.*—From neutral solns ether extracts carotin, xanthophyll (the pigments found in leaves, fats and oils, egg yolk, carrots, etc.), the coloring matter of tomatoes and paprika, and green chlorophyll. The coloring matter remains in the ether soln on shaking with normal  $NaOH$  soln or normal  $HCl$ , no apparent change taking place, altho chemically the substances may be altered more or less by this treatment.

(b) *By extraction with ether from acid solns.*—From slightly acid solns ether extracts very readily and completely the coloring matter of alkanet, annatto, turmeric, and the red dyewoods, sandalwood, camwood, and barwood. It extracts in large proportions the flavone coloring matters of fustic, Persian berries and quercitron (after hydrolysis), as well as the coloring matter of Brazilwood and the green derivatives formed from chlorophyll by alkaline treatment. It extracts in relatively small quantity the coloring matters of logwood, orchil, saffron, and cochineal. The coloring matters of this group are readily removed from ether by shaking with alkaline solns, but in most cases they rapidly undergo chemical change.

(c) *By extraction with amyl alcohol from acid solns.*—From slightly acid solns amyl alcohol extracts the major part of the coloring matters of logwood, orchil, saffron, and cochineal. Amyl alcohol extracts in relatively small proportions caramel and the anthocyanins constituting the red coloring matter of the most common fruits.

#### IDENTIFICATION

##### 21.15 *I. By Color Changes Produced with Various Reagents*

Evaporate to dryness the ether solns obtained under 21.14(a) and 21.14(b), warm the residue with a little alcohol, and dilute with  $H_2O$ . Dilute the amyl alcohol soln obtained under 21.14(c) with gasoline (b. p. 90–120°) and extract with  $H_2O$ . To portions of these somewhat purified solns of the coloring matter apply the reagents in the following manner:

*Hydrochloric acid.*—Add to soln first 1 or 2 drops of strong HCl, then excess equal to 3–4 times volume of soln.

*Sodium or potassium hydroxide.*—Make soln slightly alkaline by adding a drop of 10% NaOH or KOH soln.

*Sodium hyposulfite.*—Add a small crystal of  $Na_2S_2O_4$ .

*Ferric chloride.*—Add a small quantity of freshly prepared 0.5%  $FeCl_3$  soln very carefully, a small drop at a time, as the colorations are not obtained in some cases when an excess is used.

*Alum.*—Add to test soln  $\frac{1}{2}$  its volume of 10% K- or  $NH_4$ -alum soln.

*Uranium acetate.*—Add 5% U acetate soln dropwise.

*Sulfuric acid on dry color.*—Evaporate small quantity of soln or of the coloring matter in porcelain dish. Cool thoroly and treat dry residue with 1 or 2 drops of cold  $H_2SO_4$ . The colorations are in some cases extremely transitory, and they may be observed only the instant the acid wets the residue.

Table 2 shows the behavior of certain of the natural coloring matters when treated in the manner described above.

##### 21.16

##### *II. By Special Tests*

(a) *Chlorophyll.*—The “brown phase reaction” (20) may be useful for the characterization of chlorophyll, when this has not been previously treated with alkalies. Treat the green ether or petroleum benzene soln of the coloring matter with a small quantity of 10% soln of KOH in methyl alcohol. Color becomes brown, quickly returning to green.

(b) *Annatto* (21).—Pour on moistened filter an alkaline soln of color obtained by shaking out the oil or melted and filtered fat with warm 2% NaOH soln. If annatto is present, filter paper will absorb color, so that when washed with gentle stream of  $H_2O$  it will remain dyed a straw color. Dry filter, add a drop of  $SnCl_2$  soln, and again dry carefully. If color turns purple, presence of annatto is confirmed.

TABLE 2.—*Reaction of certain natural coloring matters to common reagents*

COLORING MATTER	STRONG HYDROCHLORIC ACID	10 PER CENT SODIUM HYDROXIDE SOLUTION	SODIUM HYPO-SULFITE	0.5 PER CENT FERRIC CHLORIDE SOLUTION	10 PER CENT ALUM SOLUTION	5 PER CENT URANIUM ACETATE SOLUTION	CONCENTRATED SULFURIC ACID ON DRY COLOR
Logwood	Deep red with excess of acid	Violet to violet-blue	Almost decolorized, color returning imperfectly by reoxidation	Dark shades of violet, brown or black (the first hue often evanescent)	Rose-red (change rather slow)	Violet, quickly fading	Red, changing to yellow
Red woods (Brazilwood, Sandalwood, Camwood and Barwood)	Deep red with excess of acid	Violet-red	.....	Dark shades of violet, brown or black (the first hue often evanescent)	Rose-red (change rather slow)	.....	.....
Anthocyanins of red fruit colors	.....	Change to green, dull blue or slate color, usually very quickly becoming browner by oxidation	Anthocyanidins derived by hydrolysis, almost completely decolorized	.....	.....	.....	.....
Alkanet	.....	Deep blue	.....	.....	.....	Yellowish green	Violet-blue
Orebil	Little or no change	Blue	Decolorized, color returning when shaken with air. Reaction more easily seen in alkaline solution	.....	.....	.....	Violet-blue
Cochineas	Little or no change	Violet	No marked change	Slightly darker	.....	Green	.....
Annatto	Remains orange. Little change	.....	Little affected	No marked change. Perhaps somewhat browner	.....	.....	Blue
Turmeric (solution in ether or alcohol characterized by pure yellow color and light green fluorescence)	Orange-red or carmine-red on addition of several volumes of concentrated acid	Orange-brown	Little affected	No marked change. Perhaps somewhat browner	Little change	Somewhat browner	Red
Flavone colors of fustic, Persian berries, quercitron, etc.	Becomes intensely yellow with 2-4 volumes of concentrated acid	Bright yellow	Little affected	Olive-green or black colorations	More strongly yellow; fustic, developing a green fluorescence	Orange colorations	Yellow to orange
Saffron	Little or no change	Remains yellow	Little affected	No marked change. Perhaps somewhat browner	Little change	Not affected	Blue
Carotin and Xanthophyll	Little change. Perhaps slightly paler	Little or no change	Little affected	.....	.....	.....	Blue, reaction obtained with difficulty
Green Chlorophyll	More brownish	"Brown phase reaction," 21.16(a)	.....	.....	.....	.....	.....
Caramel	Little or no change	Little change or slightly deeper brown	Slightly paler	No change	.....	.....	.....

(c) *Turmeric*.—Treat aqueous or dilute alcoholic soln of color with HCl until shade just begins to appear slightly orange. Divide mixture into two parts and add some  $\text{H}_3\text{BO}_3$  powder or crystals to one portion. Marked reddening will be quickly apparent, best seen by comparison with portion to which the  $\text{H}_3\text{BO}_3$  has not been added. Test may also be made by dipping piece of filter paper in alcoholic soln of coloring matter, drying at  $100^\circ$ , then moistening with weak soln of  $\text{H}_3\text{BO}_3$  to which a few drops of HCl have been added. On drying again, cherry-red color will be developed.

(d) *Cochineal*.—When presence of cochineal is suspected, acidify mixture with  $\frac{1}{2}$  its volume of HCl and shake with amyl alcohol. Wash amyl alcohol soln of coloring matter 2–4 times with equal volumes of  $\text{H}_2\text{O}$  to remove HCl, etc. Dilute the amyl alcohol with 1–2 volumes of gasoline and shake with a few small portions of  $\text{H}_2\text{O}$  to remove color. Divide combined aqueous extracts into 2 portions. To first add, dropwise, 5% U acetate soln, shaking thoroly after each addition. In presence of cochineal a characteristic emerald-green color is produced (§2). Green coloration with U salts is not developed in presence of much free acid. Therefore, add a little Na acetate before making this test, or a correspondingly large quantity of U acetate must be added. To second portion add 1 or 2 drops of  $\text{NH}_4\text{OH}$ ; in presence of cochineal, violet coloration results. This, however, is not so characteristic as first test, as many fruit colors give almost identical reactions. Cochineal is not decolorized by  $\text{Na}_2\text{S}_2\text{O}_4$  either in an acid, neutral or alkaline soln (differs from orchil).

As cochineal lakes often contain Sn, further examination for this metal should always be made when  $\text{H}_2\text{O}$ -insoluble cochineal compounds seem to be present.

(e) *Orchil*.—This coloring matter is either sulfonated or unsulfonated. Unsulfonated orchil is readily extracted by amyl alcohol from a weak acid soln, while extraction of sulfonated color is incomplete even from a strongly acidified soln. Behavior of color towards acids and alkalies is similar to cochineal, e.g., HCl produces a yellow shade and alkalies produce a bluish shade.  $\text{Na}_2\text{S}_2\text{O}_4$  reduces orchil, but color is restored by air oxidation (differing from cochineal). The characteristic property of orchil is to dye, strip, and redye wool readily.

(f) *Caramel*.—A number of tests have been developed for this coloring matter, most of them being based upon the insolubility in ether,  $\text{CHCl}_3$ , or amyl alcohol. Probably the most sensitive test is the Woodman-Newhall (§3) modification of Amthor's test with a slight deviation. To 10–20 ml of a neutral soln of the color in small centrifuge tube add 2 ml of 5%  $\text{ZnCl}_2$  and 2 ml of 2% KOH soln, stir well, and centrifuge. Pour off liquid, and to magma add 25 ml of boiling  $\text{H}_2\text{O}$ . Mix, centrifuge, and pour off liquid. Repeat this operation until aqueous wash liquor is colorless. Dissolve precipitate with 15 ml of 10% acetic acid, concentrate, neutralize carefully, and filter. Divide into 2 portions. To one add 3–5 volumes of paraldehyde in 50 ml glass-stoppered cylinder, and just sufficient absolute alcohol to form a homogeneous soln (avoid excess). Caramel will be indicated by formation of brownish precipitate on standing. To other portion of caramel soln add an equal volume of freshly prepared reagent consisting of phenylhydrazin hydrochloride, 2 parts; Na acetate, 3 parts;  $\text{H}_2\text{O}$ , 20 parts. Dark brown precipitate is formed in presence of caramel.

#### COMMERCIAL COAL TAR COLORS (§4)

The regulations for listing and certifying coal tar colors, promulgated under the Federal Food, Drug, and Cosmetic Act of 1938 (Fed. Reg., 4, 79, 3936 [1939]), contain a new nomenclature for coal tar colors permitted for use in foods, drugs, and cosmetics. The food color names and the names of the drug and cosmetic colors whose analysis is described in this chapter are:



<i>New Name</i>	<i>Former Name</i>
FD&C Blue No. 1	Brilliant Blue FCF
FD&C Blue No. 2	Indigotine
FD&C Green No. 1	Guinea Green B
FD&C Green No. 2	Light Green SF Yellowish
FD&C Green No. 3	Fast Green FCF
D&C Green No. 5	Alizarin Cyanine Green F
D&C Green No. 7	Acid Fast Green
FD&C Orange No. 1	Orange I
FD&C Orange No. 2	Orange SS
D&C Orange No. 3	Orange G
D&C Orange No. 4	Orange II
FD&C Red No. 1	Ponceau 3R
FD&C Red No. 2	Amaranth
FD&C Red No. 3	Erythrosine
FD&C Red No. 4	Ponceau SX
FD&C Red No. 32	Oil Red XO
D&C Red No. 8	Lake Red C
D&C Red No. 31	Brilliant Lake Red R
D&C Red No. 39	Alba Red
FD&C Yellow No. 1	Naphthol Yellow S
FD&C Yellow No. 2	Naphthol Yellow S-Potassium salt
FD&C Yellow No. 3	Yellow AB
FD&C Yellow No. 4	Yellow OB
FD&C Yellow No. 5	Tartrazine
FD&C Yellow No. 6	Sunset Yellow FCF
D&C Yellow No. 7	Fluorescein

No doubt the increased interest in coal tar colors created by the Act will effect a revision of analytical methods. Pending such revision the colors are referred to in this chapter by their old names.

## 21.17

## PREPARATION OF SAMPLE

Thoroughly mix and without interruption weigh out portions required. If weighing cannot be made directly into dish in which determination is to be made, use weighing bottles for this purpose, placing in each a quantity approximating weight called for, and weigh immediately.

## 21.18

## MOISTURE

(a) *Amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, naphthol yellow S and its K salt, sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine.*—Weigh ca 2 g of sample in weighed Al dish 2" in diam. or in weighing bottle of about the same diameter, dry in air oven at 135° for 6 hours or overnight, cool over H<sub>2</sub>SO<sub>4</sub> in desiccator, and weigh. Heat again 1 hour, cool in desiccator, and weigh. Repeat heating and weighing at hour intervals until weight becomes constant. Report loss in weight as moisture.

(b) *For yellow AB, yellow OB, orange SS, and oil red XO.*—Proceed as directed under (a), heating dye to 80° instead of to 135° (100° for orange SS and oil red XO)

## WATER-INSOLUBLE MATTER

## 21.19

## APPARATUS

*Prepared Gooch crucible.*—Digest a good grade of retentive asbestos with HCl (1+3), wash free from acid, and elutriate to remove fine particles. Prepare a well

packed asbestos mat of suitable thickness in the Gooch, wash with hot  $H_2O$ , dry, ignite, rewash, dry at  $135^\circ$ , cool in desiccator, and weigh. Repeat washing, heating, and drying until constant weight is obtained.

## 21.20

## DETERMINATION

(a) *Amaranth, ponceau SX, erythrosine, naphthol yellow S, sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, and brilliant blue FCF.*—Dissolve 5 g of dye in 200 ml of hot  $H_2O$  and allow soln to cool to room temp. Filter thru prepared Gooch crucible, wash with cold  $H_2O$  until all dissolved dye has been removed, dry at  $135^\circ$ , cool in desiccator, and weigh. Report increase in weight as total insoluble matter.

(b) *Ponceau 3R, orange I, and indigotine.*—Dissolve 5 g of dye in hot  $H_2O$ , using 250 ml for ponceau 3R and orange I, and 500 ml for indigotine. Cool soln to room temp., let stand overnight, and filter with moderate suction. Wash with cold  $H_2O$ , dry, cool, and weigh as directed under (a).

## 21.21

## NON-VOLATILE WATER-INSOLUBLE MATTER

*Amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, naphthol yellow S, sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine.*—Incinerate Gooch containing total insoluble matter, 21.20, at low red heat until all organic matter has been volatilized. Cool in desiccator and weigh.

## SODIUM CHLORIDE

## 21.22

## REAGENTS

All reagents must be halogen free.

*Sulfur dioxide soln.*—Saturate ice-cold  $H_2O$  with  $SO_2$ . Keep soln stoppered and in cold place.

## 21.23

## DETERMINATION

(a) *Amaranth, ponceau 3R, ponceau SX, orange I, sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine.*—Thoroughly mix 5 g of dye with 4–6 g of  $K_2CO_3$  or  $Na_2CO_3$  in 50 ml Pt, Ni, or porcelain crucible and moisten with  $H_2O$  or 50% alcohol. Cover evenly with ca 1 g of powdered carbonate, dry, and ignite at low red heat until organic matter is destroyed. Allow to cool and add enough  $H_2O$  to form thin paste. Break up any lumps present with glass rod to assure uniform suspension. Wash mixture into 250 ml volumetric flask with 100–150 ml of hot  $H_2O$  and allow to stand until all soluble salts are dissolved and mixture is cold. Dilute to mark with  $H_2O$ , mix thoroughly, and filter thru dry paper.

Place 200 ml portion of filtrate in 600 ml beaker and add enough 6–7%  $KMnO_4$  soln to oxidize sulfides and produce permanent pink color. Add ca 50 ml of  $H_2O$  and slight excess of 10%  $AgNO_3$  soln (6–8 ml usually sufficient). Partially cover beaker with watch-glass and acidify soln by carefully adding ca 12 ml of  $HNO_3$ . Heat nearly to boiling, then add the saturated  $SO_2$  soln. Boil until any excess of  $SO_2$  is removed, cool, filter thru weighed Gooch crucible, wash precipitate of  $AgCl$  with  $HNO_3$  (1+99) and then twice with  $H_2O$ , dry crucible and its contents at  $135^\circ$ , cool in desiccator, and weigh. Calculate to percentage of  $NaCl$ .

(b) *Erythrosine.*—In 500 ml volumetric flask dissolve 5 g of the dye in 400 ml of  $H_2O$ . Precipitate color acid by adding mixture of 2 ml of  $HNO_3$  and 10–20 ml

of  $\text{H}_2\text{O}$ , dilute to 500 ml, mix, and filter thru dry paper. Treat 200 ml of filtrate with slightly more 10%  $\text{AgNO}_3$  soln than is required to precipitate halogens present, add 5 ml of  $\text{HNO}_3$ , and heat to boiling. Cool, collect precipitate in weighed Gooch crucible, wash, dry, and weigh as directed under (a). If  $\text{NaI}$  is present, determine as directed under 21.54, and subtract weight of  $\text{AgI}$  from weight of precipitate. Calculate percentage of  $\text{NaCl}$  from net  $\text{AgCl}$ .

(c) *Naphthol yellow S*.—Dissolve 5 g of dye in ca 400 ml of  $\text{H}_2\text{O}$  in 500 ml volumetric flask. Precipitate most of color with  $\text{KOH}$  (ca 30% soln), make to volume, mix, and filter. Neutralize 200 ml of filtrate, make slightly acid with  $\text{HNO}_3$ , and precipitate  $\text{Cl}$  by adding a slight excess of 10%  $\text{AgNO}_3$  soln. Boil for few minutes, cool, and filter thru weighed Gooch crucible. Wash, dry, weigh precipitate and calculate as directed under (a).

(d) *Naphthol yellow S, K salt*.—Dissolve 5 g of dye in ca 800 ml of  $\text{H}_2\text{O}$  in 1000 ml volumetric flask by heating on steam bath. Cool, and precipitate most of color with  $\text{KOH}$  (ca 30% soln). Dilute to mark and filter. To 400 ml of filtrate add  $\text{HNO}_3$  until slightly acid, heat to boiling, and precipitate  $\text{Cl}$  with 10%  $\text{AgNO}_3$  soln. Boil for few minutes, cool, and filter thru weighed Gooch crucible. Wash, dry, and weigh precipitate and calculate as directed under (a).

## 21.24

### SODIUM SULFATE

(a) *Amaranth, ponceau 3R, ponceau SX, orange I, sunset yellow FCF, and indigotine*.—Transfer to 250 ml volumetric flask a volume of  $\text{H}_2\text{O}$  soln that contains 5 g of dye; add  $\text{H}_2\text{O}$ , if necessary, to bring to 200 ml; and heat on steam bath. Add pulverized C. P.  $\text{NaCl}$  as follows: For amaranth, tartrazine, and sunset yellow FCF, 70 g; for ponceau 3R, ponceau SX, orange I, indigotine, 50 g. Stopper flask and shake at frequent intervals for 1 hour. (To hasten precipitation soln may be cooled in ice  $\text{H}_2\text{O}$ .) Dilute to mark with saturated C. P.  $\text{NaCl}$  soln, shake, and filter on dry 18 cm paper. To 100 ml of filtrate add 200 ml of  $\text{H}_2\text{O}$  and 1 ml of  $\text{HCl}$  (1+9), heat to boiling, and add slight excess of hot 2%  $\text{BaCl}_2$  soln. Allow to stand overnight, filter thru weighed Gooch crucible, wash precipitate of  $\text{BaSO}_4$  thoroly with hot  $\text{H}_2\text{O}$ , dry, ignite, cool in desiccator, and weigh. Calculate weight of  $\text{Na}_2\text{SO}_4$  equivalent to  $\text{BaSO}_4$  obtained.

(b) *Erythrosine*.—Use 200 ml aliquot free of color acid, 21.23(b). Precipitate and determine the  $\text{BaSO}_4$  as directed under (a).

(c) *Light green SF yellowish, Guinea green B, fast green FCF, and brilliant blue FCF*.—Transfer to 250 ml flask volume of soln that contains 5 g of dye; add  $\text{H}_2\text{O}$ , if necessary, to bring to ca 200 ml, and heat on steam bath. Add 5 g of phosphotungstic acid and shake at intervals until dissolved. Then add 50 g of pure pulverized  $\text{NaCl}$ , shaking at intervals to dissolve the salt. Cool, dilute to mark with saturated pure  $\text{NaCl}$  soln, shake, and filter. To 100 ml of filtrate add 200 ml of  $\text{H}_2\text{O}$  and 1 ml of  $\text{HCl}$  (1+9) and determine  $\text{Na}_2\text{SO}_4$  as directed under (a).

(d) *Naphthol yellow S*.—Use 200 ml of filtered soln, 21.23(c), neutralize, and make acid with  $\text{HCl}$ . Precipitate and determine  $\text{Na}_2\text{SO}_4$  as directed under (a).

(e) *Naphthol yellow S, K salt*.—Use 400 ml aliquot of filtered soln, 21.23(d), make acid with  $\text{HCl}$ , and determine  $\text{Na}_2\text{SO}_4$  as directed under (a).

## 21.25

### SODIUM ACETATE

*Brilliant blue FCF*.—Weigh 10 g of dye into 200 ml Kjeldahl flask, add 25 ml of  $\text{H}_2\text{O}$ , and connect flask in upright position to vertical straight-tube water-jacketed condenser. Insert separator or dropping funnel thru stopper of flask, together with tube leading from flask to condenser. Add 15 ml of  $\text{H}_3\text{PO}_4$  (sp. gr. 1.7) and heat contents of flask to boiling. Collect acetic acid and condensed steam in 300 ml Erlen-

meyer flask containing standard 0.5 N NaOH soln. Continue boiling until ca 250 ml has been distilled over, replacing distillate by  $H_2O$  from dropping funnel so that volume in Kjeldahl flask remains ca 20 ml. (This distillation should require ca 2 hours.) Remove receiver and titrate excess alkali with standard 0.5 N HCl, using phenolphthalein indicator. Run blank distillation and deduct resulting acidity found. From corrected acidity calculate quantity of Na acetate present in dye.

**21.26****SULFATED ASH**

Weigh accurately in weighing bottle ca 5 g of dye and transfer to Pyrex Kjeldahl flask or tall beaker, washing out weighing bottle with a little  $H_2O$ . Destroy organic matter to convenient extent by digestion, using 15 ml of  $H_2SO_4$ , and adding  $HNO_3$  as required. As bulk of  $HNO_3$  is driven off, lower flame to avoid reaction on glass. Transfer mixture to weighed Pt dish and heat over ring burner, using at first a low flame at safe distance below dish, increasing flame, and bringing it closer to dish by gradual steps. Thus continue destruction of organic matter and volatilization of acids. Continue heating until production of acid fumes decreases. If C remains, remove flame, let mass cool a little, and add  $H_2SO_4$  dropwise until mass is moistened. Repeat treatment until C is burned off and ash is white or reddish. Heat carefully with blast lamp until fusion takes place with production of clear liquid free from bubbles. Cool in desiccator and weigh. After deducting weight of  $Na_2SO_4$  equivalent to inorganic Na salts (chlorides, sulfates, carbonates, etc.) found in other determinations, calculate to percentage of metallic Na combined in dye.

**21.27****HEAVY METALS**

Moisten sulfated ash obtained under 21.26 with few ml of HCl and evaporate to dryness on steam bath. Warm residue with 20 ml of HCl (1+19) until all soluble material has dissolved, transfer to 100 ml volumetric flask, dilute to 100 ml, mix, and filter thru dry paper. Reserve two 40 ml aliquots for determination of Al, Ca, Fe, and Mg. Pour 20 ml of filtrate into test tube and pass in washed stream of  $H_2S$  for 30 min. No turbidity other than that due to precipitated S should appear. If colored precipitate is formed, filter and test it for Cu and Sn.

**21.28****LEAD (ss)**

(Applicable to all permitted dyes)

Place 5 g of dye in tall-form 500 ml Pyrex beaker, cover with watch-glass, add 15 ml of  $HNO_3$ , and let boil (or heat gently) till rapid evolution of brown fumes has ceased. Add 15 ml of  $H_2SO_4$  and continue heating. Add small quantities (1-2 ml) of  $HNO_3$  at intervals until organic matter is destroyed and soln is colorless or at most pale yellow. Continue heating, with evolution of dense white fumes, until very small quantity (3-5 ml) of soln remains in beaker. Cool soln, and add 15-20 ml of  $H_2O$ . Re-evaporate soln thus formed to white fumes, cool, take up in 100 ml of  $H_2O$ , add 100 ml of alcohol, and let stand overnight. Filter out precipitate of  $PbSO_4$ , which may be present in such small quantity as to escape detection with naked eye, and wash thoroly with 50% alcohol (ca 100 ml). Two 9 cm C. S. & S No. 590 filter papers, or suitable fritted glass crucible, are satisfactory for retaining the  $PbSO_4$ .

Place filter paper in small beaker, add 20 ml of 40%  $NH_4$  acetate soln, and heat to boiling, breaking up paper with glass rod. Filter thru C. S. & S. No. 590 9 cm paper, or thru fritted glass crucible, into 100 ml colorimeter tube and wash with 4%  $NH_4$  acetate soln until 50 ml mark is reached. When filter paper is used for retaining the  $PbSO_4$ , and the Pb content appears to exceed 10 p.p.m. use 40 ml of 40%  $NH_4$  acetate soln for dissolving the  $PbSO_4$  instead of 20 ml, wash with 4%  $NH_4$  acetate soln, filter into 100 ml volumetric flask, make to volume, mix, and use aliquot portion in

colorimeter tube. Prepare standards containing known quantities of Pb for comparison. To these add same quantity of  $\text{NH}_4$  acetate as was used with sample and dilute all tubes to definite volume with  $\text{H}_2\text{O}$ . To each tube add 2 or 3 drops of acetic acid and 10 ml of freshly prepared  $\text{H}_2\text{S}$  water. Shake tubes to insure thoro mixing and estimate quantity of Pb by comparison with standards. Run blanks on all reagents used.

## 21.29

## IRON, ALUMINUM, CALCIUM, AND MAGNESIUM (86)

(Applicable to all permitted dyes)

To one of the two portions reserved under 21.27, add 5 g of  $\text{NH}_4\text{Cl}$  and neutralize with  $\text{NH}_4\text{OH}$  (1+1), boiling to drive off any excess. If precipitate is very slight, it may be disregarded; otherwise, filter thru quantitative paper, wash with  $\text{H}_2\text{O}$  containing trace of  $\text{NH}_4\text{OH}$  (reserving filtrate and washings), and ignite paper and precipitate in weighed crucible. Weigh mixture of  $\text{Fe}_2\text{O}_3$  and  $\text{Al}_2\text{O}_3$ . Place mixed oxides in 500 ml Erlenmeyer flask and dissolve in aqua regia, boiling to drive off Cl. Add  $\text{H}_2\text{O}$  to bring volume to ca 75 ml and add  $\text{NH}_4\text{OH}$  to incipient precipitation. Dissolve precipitate with as little  $\text{HCl}$  as possible, cool, and titrate the ferric Fe present with 0.1 N  $\text{TiCl}_3$  soln, 21.36, using 5 g of  $\text{NH}_4\text{CNS}$  as indicator. Calculate the Fe as  $\text{Fe}_2\text{O}_3$ . To calculate quantity of  $\text{Al}_2\text{O}_3$ , deduct weight of  $\text{Fe}_2\text{O}_3$  from total weight of mixed oxides. From weight of oxide calculate percentage of metallic Al. Pass washed stream of  $\text{H}_2\text{S}$  into alkaline filtrate from the Fe- and Al-hydroxides. White precipitate indicates presence of Zn.

To other reserved portion add 250 ml of  $\text{H}_2\text{O}$  to insure low concentration of Mg, if present. Heat to boiling and add 3.5 g of  $\text{NH}_4\text{Cl}$  and enough  $\text{NH}_4\text{OH}$  soln (1+99) to make soln barely alkaline. Filter off precipitated hydroxides of Fe and Al. Wash and discard precipitate. Heat combined filtrate and washings to boiling and add 1 g of  $\text{NH}_4$  oxalate. After cooling and letting stand for an hour, filter thru asbestos mat prepared on small Witt plate in glass funnel and wash with very little  $\text{H}_2\text{O}$ , reserving combined filtrate and washings. Place mat in beaker, add 100 ml of  $\text{H}_2\text{O}$  and 2 ml of  $\text{H}_2\text{SO}_4$ , heat gently until Ca oxalate dissolves, and titrate with 0.1 N  $\text{KMnO}_4$  soln. Calculate as Ca.

Heat to boiling reserved filtrate and washings and add N  $\text{NaNH}_2\text{HPO}_4$  soln until there is no further precipitation. While stirring add ca  $\frac{1}{2}$  the volume of  $\text{NH}_4\text{OH}$  (1+9). Let stand 3 hours, filter thru ashless paper, and wash with  $\text{NH}_4\text{OH}$  (1+49). Ignite filter and precipitate in weighed crucible, cool in desiccator, and weigh the  $\text{Mg}_3\text{P}_2\text{O}_7$ . Calculate as Mg.

## 21.30

## ARSENIC—See 29.1–29.5

## ETHER EXTRACTIVES

## 21.31

## REAGENT

*Washed ether.*—Wash 1 liter of ether with 3 successive 150 ml portions of  $\text{H}_2\text{O}$  immediately before using.

## 21.32

## DETERMINATION

(a) *Amaranth, ponceau 3R, ponceau SX, orange I, naphthol yellow S, sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine.*—Place in separator volume of soln containing 10 g of dye and add  $\text{H}_2\text{O}$ , if necessary, to bring volume to 200 ml, and, in the case of indigotine, to 500 ml. Extract with 2 successive 100 ml portions of the washed ether, shaking 1 min. during each extraction. Remove ether by decantation into clean separator and

rinse first separator with 5 ml of ether, decanting into second separator. Reserve color soln. Wash combined extracts with 20 ml portions of  $H_2O$  until washings are colorless. Decant ether into beaker, rinse separator with 5 ml of ether, and decant into same beaker. Place beaker in dust-free atmosphere, allow ether to evaporate to volume of 50 ml, and transfer to weighed flat-bottomed 100 ml dish, previously dried to constant weight over  $H_2SO_4$  in desiccator. Rinse beaker with 5 ml of ether and drain into same dish. Let remainder of ether evaporate and dry over  $H_2SO_4$  to constant weight. Result represents the neutral extract.

To reserved color soln, add 2 ml of 10% NaOH soln and extract and rinse with ether. Reserve color soln. Wash combined ether extracts and rinsings with 20 ml portions of 0.1 N NaOH soln until washings are colorless. Evaporate ether, dry, and weigh. Result represents the alkaline extract.

To the color soln reserved from the alkaline extraction, add twice the volume of HCl (1+3) necessary to neutralize. Repeat previous procedure, but do not reserve color soln. Wash ether extract with 0.1 N HCl until washings are colorless. Result represents the acid extract.

(b) *Erythrosine*.—Determine as directed under (a), omitting acid extraction. In case of neutral extraction, wash combined ether extracts with three 20 ml portions of  $H_2O$ .

(c) *Naphthol yellow S, K salt*.—Proceed as directed in (a) but dissolve 5 g of dye in 500 ml of  $H_2O$ .

### 21.33 ISOPROPYL ETHER EXTRACTIVES (#7)

*Lake red C*.—Transfer 5 g sample to cellulose thimble and extract with isopropyl ether for 2 hours in Dunbar extractor. Pour extract into weighed flat-bottomed 100 ml dish, rinse extractor with 10 ml of isopropyl ether and drain into same dish. Evaporate ether at ca  $40^\circ$ , and dry residue over  $H_2SO_4$  to constant weight ( $\pm 0.5$  mg). Designate weight obtained as "a".

Re-extract original sample for 2 additional hours, evaporate, dry, and weigh as above. Designate this weight as "b". Isopropyl ether extract = a - b.

### 21.34 PETROLEUM BENZINE EXTRACTIVES (#7)

*Alba red*.—Weigh 5 g into Soxhlet thimble, cover with wad of cotton (to diffuse solvent), place in Dunbar extractor and extract with petroleum benzine for 1 hour. Transfer extract to weighed crystallizing dish, allow to evaporate spontaneously, dry in desiccator overnight, and weigh residue. Report per cent petroleum benzine extract.

### 21.35

### SULFUR

*Amaranth, ponceau SR, ponceau SX, orange I, naphthol yellow S (and its K salt), sunset yellow FCF, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and*

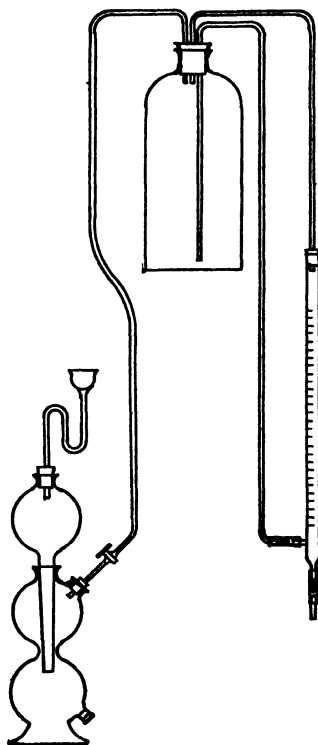


FIG. 27.—APPARATUS FOR TITRATION WITH TITANIUM TRICHLORIDE

**indigotine.**—Place ca 0.2 g of sample in Parr calorimetric bomb and mix thoroly with ca 10 g of  $\text{Na}_2\text{O}_2$ . Add a few mg of S-free sugar if necessary to aid in igniting the mass. Close bomb and ignite. When cool, open bomb, place in 600 ml beaker, and cover beaker with watch-glass. Dissolve residue by adding warm  $\text{H}_2\text{O}$  thru lip of beaker until bomb is covered. Acidify soln cautiously with  $\text{HCl}$  and filter if necessary. Determine  $\text{BaSO}_4$  as directed under 21.24(a), beginning "heat to boiling, and add slight excess of hot 2%  $\text{BaCl}_2$  soln." Deduct the S equivalent to the  $\text{Na}_2\text{SO}_4$  determined under 21.24.

## COLOR ACID AND DYE

### *I. By Titration with Titanium Trichloride*

#### 21.36

#### REAGENT

**Standard titanium trichloride soln.**—To 200 ml of the commercial 15%  $\text{TiCl}_3$  soln, add 150 ml of  $\text{HCl}$  and dilute to 2 liters. Make soln ca 0.1 *N*, place in container with  $\text{H}$  atmosphere provision (28), and allow to stand 2 days for absorption of residual  $\text{O}$ .

#### 21.37

#### STANDARDIZATION OF SOLUTION

**Method I.**—Prepare liter of 0.1 *N*  $\text{Fe}_2(\text{SO}_4)_3$  by dissolving ingot  $\text{Fe}$ , Bureau of Standards Sample 55, in 30 ml of  $\text{H}_2\text{SO}_4$ . Dilute to ca 400 ml, adding slowly, with stirring, a soln of pure  $\text{KMnO}_4$  (3.16 g dissolved in ca 200 ml  $\text{H}_2\text{O}$ ) until faint but perceptible reddish tint results. The last few ml should be added dropwise. Cool and dilute to 1 liter. Measure 20 ml of the 0.1 *N*  $\text{Fe}_2(\text{SO}_4)_3$  into 500 ml flask, pass in strong stream of  $\text{CO}_2$ , and add the  $\text{TiCl}_3$  soln rapidly until near end point. Add 5 g of pure  $\text{NH}_4\text{CNS}$  and resume addition of  $\text{TiCl}_3$  carefully until the red color just disappears.

**Method II.**—Make up 0.1 *N*  $\text{KMnO}_4$  and standardize carefully, using  $\text{Na}$  oxalate, Bureau of Standards Sample 40, according to directions supplied with sample. Weigh 3 g of  $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$  and transfer to 500 ml flask. Introduce stream of  $\text{CO}_2$  and add 50 ml of recently boiled  $\text{H}_2\text{O}$  and 25 ml of 40% (by weight)  $\text{H}_2\text{SO}_4$ . Then, without interrupting current of  $\text{CO}_2$ , add rapidly 40 ml of the standardized  $\text{KMnO}_4$  soln. Add the  $\text{TiCl}_3$  soln until near calculated end point. Then add quickly 5 g of  $\text{NH}_4\text{CNS}$ , and complete titration. Run blank on 3 g of  $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ , using the same quantities of  $\text{H}_2\text{O}$ , acid,  $\text{NH}_4\text{CNS}$ , and the current of  $\text{CO}_2$ .

#### 21.38

#### INDICATOR

For many dyes the  $\text{TiCl}_3$  titration end point is indicated by a sharp decolorization. For some dyes the change is so gradual that an excess of  $\text{TiCl}_3$  (not more than 0.3 ml of approximately 0.1 *N* soln) is required, and a suitable standard soln of some other dye must be used for the back titration, methylene blue serving well for this purpose. In other cases it is better to use an indicator which is reduced after original dye has reacted with the  $\text{TiCl}_3$ . Thus a known quantity of light green SF yellowish serves well for this purpose.

**Yellow AB and OB, orange SS, oil red XO, and indigotine.**—Prepare a 0.5% soln; for other dyes a 1% soln.

#### 21.39

#### DETERMINATION

(a) **Amaranth, ponceau 3R, and sunset yellow FCF.**—Place in 500 ml Erlenmeyer flask a volume of soln that corresponds to ca 20 ml of 0.1 *N*  $\text{TiCl}_3$ . Add 10 g of  $\text{Na}$  citrate and  $\text{H}_2\text{O}$  if necessary to bring volume to 150 ml. Heat to boiling, introduce

stream of  $\text{CO}_2$  and titrate with standardized  $\text{TiCl}_3$ , keeping  $\text{CO}_2$  flow continuous to the end.

(b) *Orange I, ponceau SX, tartrazine, guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine.*—Proceed as directed under (a), substituting 15 g of Na acid tartrate for Na citrate.

(c) *Naphthol yellow S and its K salt.*—Proceed as directed under (b), using as indicator a volume of light green SF yellowish standardized soln (freshly made) that contains 10 mg of dye. Run a blank on the tartrate, light green SF yellowish, and  $\text{H}_2\text{O}$ .

TABLE 3.—Quantities of color acids and of pure coal-tar dye equivalent to 1 ml of 0.1 N titanium trichloride solution

DYE	MOLECULAR WEIGHT OF COLOR ACID	COLOR ACID EQUIVALENT TO 1 ML 0.1 N $\text{TiCl}_3$	DYE EQUIVALENT TO 1 ML 0.1 N $\text{TiCl}_3$
Amaranth.....	538.4	0.01346	0.01511
Ponceau 3R.....	450.4	.01126	.01236
Ponceau SX.....	436.3	.010907	.012006
Orange I.....	328.3	.008207	.008756
Naphthol yellow S.....	314.2	.002618	.002985
Naphthol yellow S-K salt.....	314.2	.002618	.003253
Sunset yellow FCF.....	408.3	.010206	.011305
Tartrazine.....	468.3	.01171	.01336
Guinea green B.....	668.5	.03342	.03453
Light green SF yellowish.....	748.7	.03743	.03963
Fast green FCF.....	764.5	.03822	.040423
Brilliant blue FCF.....	748.5	.03743	.03963
Indigotine.....	422.3	.02112	.02332

## 21.40

### II. By Precipitation (29)

*Erythrosine.*—To volume of  $\text{H}_2\text{O}$  soln of sample that contains 0.25 g of dye, add, if necessary, sufficient  $\text{H}_2\text{O}$  to bring volume to 100 ml. Add 5 ml of  $\text{HNO}_3$  of ca 0.6 N strength and filter thru weighed Gooch crucible. Wash thoroly with 0.5%  $\text{HNO}_3$  and finally with not more than 10 ml of  $\text{H}_2\text{O}$ . Do not allow precipitate to cake in crucible until washing has been completed. Dry to constant weight at  $135^\circ$ .

### PURE COAL TAR DYE

#### 21.41 I. By Direct Titration with Standard Titanium Trichloride Solution

(a) *Acid fast green (Official, first action).*—To 100 ml of 1% soln of dye in 500 ml Erlenmeyer flask add 15 g of Na acid tartrate and ca 25 ml of  $\text{H}_2\text{O}$ . Heat to boiling and titrate with the standard  $\text{TiCl}_3$  soln, 21.36, under  $\text{CO}_2$ . 1 ml of 0.1 N  $\text{TiCl}_3$  = 0.03626 g of acid fast green. Calculate percentage of pure dye.

(b) *Alba red (Official, first action).*—Pipet 100 ml of 0.2% alcoholic soln of dye into 500 ml Erlenmeyer flask, and add 15 g of Na acid tartrate and ca 25 ml of  $\text{H}_2\text{O}$ . Heat to boiling, and titrate with the standard  $\text{TiCl}_3$  soln, 21.36, under  $\text{CO}_2$ . 1 ml of 0.1 N  $\text{TiCl}_3$  = 0.00823 g of alba red. Calculate percentage of pure dye.

(c) *Alizarine cyanine green F (30)*—Dissolve quantity of color expected to consume ca 20 ml of 0.1 N  $\text{TiCl}_3$  in ca 125 ml of 50% alcohol. Add 15 g of Na citrate, heat to boiling, and titrate with the standard  $\text{TiCl}_3$  soln, 21.36, under  $\text{CO}_2$ . 1 ml of 0.1 N  $\text{TiCl}_3$  = 0.03113 g of alizarine cyanine green F. Calculate percentage of pure dye.

(d) *Brilliant lake red R and lake red C.*—In wide-mouthed Erlenmeyer flask dissolve 0.2 g of dye in 5 ml of  $\text{H}_2\text{SO}_4$ . Dilute with 100 ml of  $\text{H}_2\text{O}$  and add 30% NaOH



soln until barely acid. Add 15 g of Na acid tartrate, dissolve by heating, add ca 125 ml of alcohol, heat to boiling, and titrate with the standard  $\text{TiCl}_3$  soln, 21.36, to yellow end point. (Titrate slowly, especially toward end of titration.) 1 ml of 0.1  $N$   $\text{TiCl}_3$  = 0.007783 g of brilliant lake red R, and 0.00997 g of lake red C. Calculate percentage of pure dye.

(e) *Fluorescein (Official) (30)*.—Dissolve 0.4 g of dye in 200 ml of  $\text{H}_2\text{O}$  containing a few ml of 10%  $\text{NaOH}$ . Add 10 g of Na tartrate, heat to boiling, and titrate with the standard  $\text{TiCl}_3$  soln, 21.36, under  $\text{CO}_2$ . 1 ml of 0.1  $N$   $\text{TiCl}_3$  = 0.0166 g of fluorescein. Calculate percentage of pure dye.

(f) *Orange G (Official) (30)*.—Dissolve ca 0.2 g of dye in ca 125 ml of  $\text{H}_2\text{O}$ , add 15 g of Na acid tartrate, heat to boiling, and titrate with the standard  $\text{TiCl}_3$  soln, 21.36, under  $\text{CO}_2$ . 1 ml of 0.1  $N$   $\text{TiCl}_3$  = 0.01131 g of orange G. Calculate percentage of pure dye.

(g) *Orange II (Official, first action)*.—Proceed as directed under (a), but use 20 ml instead of 100 ml of 1% soln of dye. 1 ml of 0.1  $N$   $\text{TiCl}_3$  = 0.00876 g of orange II. Calculate percentage of pure dye.

(h) *Yellow AB, yellow OB, orange SS, and oil red XO*.—Dissolve 15 g of Na acid tartrate in 100 ml of  $\text{H}_2\text{O}$  and add 0.1 g of dye dissolved in 100 ml of alcohol. Titrate with the standard  $\text{TiCl}_3$  soln, 21.36, under  $\text{CO}_2$ , using as indicator 10 mg of light green SF yellowish from fresh standardized soln, as directed under 21.39(c). Run blank as directed under 21.39(c), including also the 100 ml of alcohol. 1 ml of 0.1  $N$   $\text{TiCl}_3$  = 0.006180 g of yellow AB, 0.006530 g of yellow OB, 0.006557 g of orange SS, and 0.00691 g of oil red XO. Calculate percentage of pure dye.

## 21.42

## II. By Precipitation

*Erythrosine*.—Multiply percentage of color acid obtained under 21.40 by factor 1.074.

## 21.43

## MATTER INSOLUBLE IN CARBON TETRACHLORIDE

*Yellow AB, yellow OB, orange SS, and oil red XO*.—In 100 ml beaker mix 5 g of the dye with 50 ml of  $\text{CCl}_4$ , stir, and heat to boiling. Wash Gooch crucible prepared as directed under 21.19 with  $\text{CCl}_4$  and heat at 100–105° to constant weight. Transfer hot dye soln and residue to crucible and wash with five 10 ml portions of  $\text{CCl}_4$ . Dry at 100–115° and weigh.

## 21.44

## WATER-SOLUBLE MATTER

*Yellow AB, yellow OB, orange SS, and oil red XO*.—Place 10 g of the well-powdered dye in 500 ml separator, add 100 ml of benzene, stopper, and mix until dissolved. Extract with two 100 ml portions of  $\text{H}_2\text{O}$ , and evaporate 100 ml of extract in weighed Pt or crystallizing dish on steam bath. Dry in oven at 100–105°, cool, and weigh. Result represents the neutral extractive. Test small portions of remainder of filtrate for chlorides, sulfates, and nitrates. If more than traces are present, make proper analyses on aliquot portions of filtrate.

## MELTING POINT

(Yellow AB, yellow OB, and orange SS)

## 21.45

## APPARATUS

The apparatus, Fig. 28, consists of tube ca 15 cm long and 3.5 cm internal diam. with bulb of 5 cm internal diameter. Fill with glycerol to about height indicated.

Fit tube with cork stopper carrying glass tube (A), 5 mm in diam., which reaches nearly to bottom of bath; an ordinary test tube (B) in which a thermometer (C) is suspended by means of rubber stopper in such manner that Hg column is wholly within tube and Hg bulb equidistant from its walls; a long stemmed thermometer (D), supported so as to reach short distance below tube (B); and an outlet tube (E) to permit escape of air and vapor.

## 21.46

## DETERMINATION

To capillary tube of 1 mm or smaller internal diam., sealed at one end, transfer small portion of sample by inserting into sample open end of capillary, removing, inverting, and gently tapping until well packed substance fills bottom of tube to height of 2–4 mm. Attach capillary tube to thermometer (C) by means of small rubber band, so that sample is placed at about middle of Hg bulb. Replace thermometer in tube, connect tube A to air blast and force fairly rapid stream of air bubbles thru bath. Raise temp. of bath rapidly to within 5° of approximate melting point of sample. Keep temp. constant until thermometer reading is within 1° of that of bath. Then raise temp. slowly until melting point is observed. On approaching within 0.5° of melting point, the substance darkens; the true melting point is indicated by formation of meniscus on upper surface. When this condition is observed, hold temp. as nearly constant as possible until whole sample has liquefied.

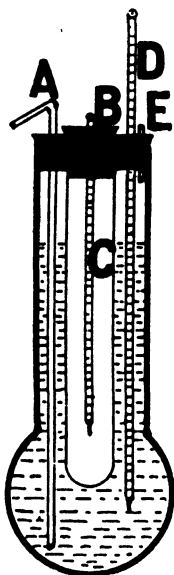


FIG. 28.—APPARATUS FOR DETERMINATION OF MELTING POINT

(a) *Amaranth, ponceau 3R, ponceau SX, sunset yellow FCF, tartrazine, and indigotine.*—Prepare H<sub>2</sub>O soln of such concentration that 50 ml will contain 0.2 g of dye.

(b) *Light green SF yellowish, fast green FCF, and brilliant blue FCF.*—Prepare H<sub>2</sub>O soln of such concentration that 10 ml will contain 0.1 g of dye.

## 21.49

## DETERMINATION

(a) *Amaranth and tartrazine.*—To 50 ml of prepared soln, 21.48(a), add 1 ml of HCl. Extract lower sulfonated dye by shaking soln successively in 3 separators, each containing 50 ml of amyl alcohol. Wash the amyl alcohol extracts by shaking successively with three 50 ml portions of 0.25 N HCl until washings are practically colorless. Dilute amyl alcohol in each separator with 100 ml portions of gasoline (b.p. 90–120°), and remove lower sulfonated dye by washing with several 10 ml volumes of H<sub>2</sub>O, passing each portion thru the 3 separators in an order the reverse of that previously followed.

Determine dye in H<sub>2</sub>O extract by titration against standard TiCl<sub>3</sub> soln, 21.36, using 10 g of Na citrate and volume of 100 ml. Run blank determination on all reagents, using 1 mg of the dye concerned. Calculate result to percentage of fast red E in

## LOWER SULFONATED DYES

## 21.47

## REAGENT

*Salt acetate soln.*—Dissolve 125 g of NaCl in H<sub>2</sub>O, add 12 ml of acetic acid and a soln of 13.6 g of Na acetate, and dilute to 500 ml.

## 21.48

## PREPARATION OF SOLUTION

amaranth and to fast yellow G in tartrazine. 1 ml of 0.1 *N*  $\text{TiCl}_3$  soln = 0.01256 g of fast red E and 0.01081 g of fast yellow G. If quantity of dye is very low, it may be determined colorimetrically, amaranth or tartrazine, as appropriate, being used as standard.

(b) *Ponceau SX and sunset yellow FCF*.—Proceed as directed under (a), substituting 5% NaCl soln for 0.25 *N* HCl, and determine quantity of dye colorimetrically by comparison with standard soln of the dye.

(c) *Ponceau 3R*.—Proceed as directed under (a), substituting mixture of equal volumes of amyl alcohol and gasoline (b.p. 90–120°) in place of the amyl alcohol and running the blank with 1 mg of ponceau 3R. Calculate result to percentage of Na trimethyl benzene-azo- $\beta$ -naphthol sulfonate, using factor 0.009807.

(d) *Indigotine*.—Proceed as directed under (a), substituting 0.25 ml of acid for 1 ml and Na acid tartrate for Na citrate, washing with 0.0625 *N* instead of 0.25 *N* acid, and running blank with 1 mg of indigotine. Calculate result to percentage of Na indigo monosulfonate, using factor 0.01821.

(e) *Light green SF yellowish, fast green FCF, and brilliant blue FCF*.—To 10 ml of prepared dye soln, 21.48(b), add 40 ml of the salt acetate soln and extract successively in 3 separators, each containing 100 ml of amyl alcohol. Wash extracts with 100 ml portions of the salt acetate soln, passing each wash portion successively thru the 3 separators in order used for original extractions. Remove dye from alcohol as directed under (a) and determine colorimetrically by comparison with a standard guinea green B soln of approximately same strength for light green SF yellowish, by comparison with a standard soln of fast green FCF and brilliant blue FCF for subsidiary dyes in the latter. Report as percentage of guinea green B in light green SF yellowish and subsidiary dye in fast green FCF or brilliant blue FCF.

## 21.50

### BOILING RANGE OF $\Psi$ CUMIDINE FROM PONCEAU 3R

(a) Dissolve 60 g of dye in 600–700 ml beaker with ca 450 ml of boiling  $\text{H}_2\text{O}$ , and add the hot soln very slowly to warm (60–80°) soln of 100 g of  $\text{SnCl}_2$  in 100 ml of HCl in tall liter beaker. Add dye soln in 10–20 ml portions, waiting after each addition until mixture is pale brown; otherwise dye will be precipitated, in which case it can be reduced only with difficulty. As reduction proceeds and soln becomes more dilute, heat to boiling, taking care that mixture does not boil over after each addition of dye, as some heat is generated by the reaction. After all the dye has been added and reduced, allow mixture to cool, and make alkaline by addition of ca 75 g of NaOH dissolved in 150–200 ml of  $\text{H}_2\text{O}$ .

Cool alkaline mixture and extract cumidine by shaking it with three 200 ml portions of ether. Combine ether extracts thus obtained and wash with  $\text{H}_2\text{O}$  until alkali and salts are removed. Evaporate solvent on steam bath, but avoid such prolonged heating as may tend to volatilize the base. Transfer residue of crude cumidine to small side-necked flask and distil it, carefully avoiding overheating. Observe range within which the substance volatilizes.

(b) Proceed as directed under (a) to directions for extraction with ether. Then steam distil the alkaline mixture until no more oil is carried over. Extract distillate with two 150 ml portions of ether. Wash combined extracts with successive 10 ml portions of  $\text{H}_2\text{O}$  until alkali and salts are removed. Evaporate solvent and complete the determination as directed under (a).

## 21.51

### ISOMERIC AND SIMILAR DYES IN AMARANTH

Take volume of  $\text{H}_2\text{O}$  soln of sample that contains 0.1 g of dye and dilute, if necessary, to 40 ml with  $\text{H}_2\text{O}$ . Add 10 ml of 0.1 *N* benzidine soln (9.2 g of base per

liter in 0.5 *N* HCl), mix well, and allow to stand exactly 2 min. Filter thru fluted paper and dilute 10 ml of filtrate to 100 ml. Compare this soln colorimetrically with standard amaranth soln containing 0.4 mg of the dye/100 ml. The soln of the amaranth to be tested may be used in making the standard soln. If, after the benzidine treatment, the soln obtained is not more intensely colored than the standard soln, the proportion of isomeric dyes may be considered to be below 1.5%.

#### BROMINE IN FLUORESCIN DERIVATIVES—OFFICIAL, FIRST ACTION

##### 21.52

##### APPARATUS

Consists of 100 ml round-bottomed flask, A, with 19/38 ST inner joint; adapter, B, with 19/38 ST inner and 10/30 ST outer joints; condenser, C, with jacket ca 130 mm long, and absorption flask with two bulbs, D. Condenser is equipped with 10/30 ST inner joints. Small dropping funnel is fused to tube above jacket. Absorption flask has outer 10/30 ST joint. Small springs (not shown) are attached to hooks on joints in order to keep apparatus tightly connected during use.

##### 21.53

##### DETERMINATION

Place sample calculated to contain 40–60 mg of Br in oxidation flask and dissolve in 2 ml of 10% NaOH soln and 8 ml of H<sub>2</sub>O (10 ml aliquot of suitable soln of dye may be used). Lubricate joints of apparatus with H<sub>3</sub>PO<sub>4</sub> and connect flask to condenser. Place ca 20 ml of 1% hydrazine sulfate soln and 5 ml of 10% NaOH soln in absorption flask and connect to apparatus.

Add thru addition tube 5 ml of CrO<sub>3</sub> soln (1+1), wash down with 2–3 ml of H<sub>2</sub>O, and then slowly add 10 ml of H<sub>2</sub>SO<sub>4</sub>. If vigorous reaction begins at this point, allow reaction to subside before heating flask; if reaction does not begin on addition of the acid, heat gently with small flame, but remove flame before reaction becomes too vigorous, as otherwise reaction mixture may foam up into condenser. When reaction subsides and most of dye is in soln, heat mixture to boiling. When foaming has subsided, add 5 ml of H<sub>2</sub>SO<sub>4</sub> thru dropping funnel, boil 10 min., add another 5 ml of H<sub>2</sub>SO<sub>4</sub>, and boil again for 10 min. Drain H<sub>2</sub>O from condenser and boil reaction mixture 5 min. to drive all liberated halogen into absorber.

Disconnect absorption flask and wash contents into 500 ml I flask. Add ca 12 ml of H<sub>3</sub>PO<sub>4</sub>, 5 ml of 3% KCN soln, and 10 ml of 3% KMnO<sub>4</sub> soln. Stopper flask and mix contents by gentle swirling, wetting entire inside surface. Let stand at least 7 min., then add solid FeSO<sub>4</sub>·(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>·6H<sub>2</sub>O to destroy all KMnO<sub>4</sub> and MnO<sub>2</sub> (2 g excess does no harm).

Add ca 2 g of KI and titrate liberated I with 0.05 *N* Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln, using starch indicator. (End point is disappearance of starch—I color; take care to avoid over-titration, as color of soln remains light blue.) 1 ml of 0.05 *N* Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> = 0.001998 g of Br.

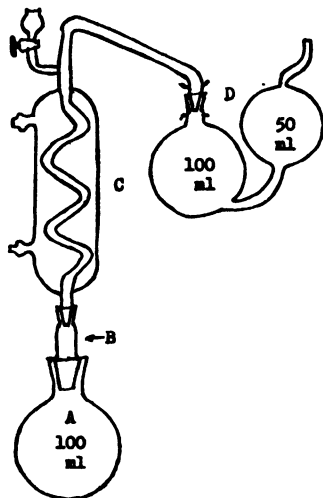


FIG. 29.—APPARATUS FOR BROMINE DETERMINATION

## 21.54

## SODIUM IODIDE

*Erythrosine*.—Dilute to ca 400 ml a volume of  $H_2O$  soln of sample that contains 5 g of dye and add mixture of 2 ml of  $HNO_3$  and 10–20 ml of  $H_2O$ . Dilute to exactly 500 ml, mix, and filter thru dry paper. Place 200 ml of filtrate in porcelain casserole and make slightly alkaline with 10%  $NaOH$  soln. Add ca 20 ml of 7%  $KMnO_4$  soln, mix, and add 10 ml of  $HNO_3$ . Place on steam bath and evaporate to dryness. Add 5 ml of 7%  $KMnO_4$  soln and 5 ml of  $HNO_3$  and again evaporate to dryness. Then add ca 50 ml of  $H_2O$ , 5 ml of  $HNO_3$ , and 25–30 ml of a saturated  $SO_2$  soln. Stir frequently, breaking up any lumps, until hydrated oxide of Mn has dissolved. Filter, wash paper with  $H_2O$ , add to combined filtrate and washings an excess of 10%  $AgNO_3$  soln, and boil until  $SO_2$  has been expelled. Collect precipitate on weighed Gooch crucible, wash first with  $HNO_3$  (1+99), then twice with  $H_2O$ , dry, and weigh. Calculate as percentage of  $NaI$ .

## 21.55

## IODINE ORGANICALLY COMBINED

*Erythrosine*.—Place in porcelain casserole a volume of  $H_2O$  soln of sample that contains 0.3–0.4 g of dye. Add 5 ml of 10%  $NaOH$  soln and 35 ml of 7%  $KMnO_4$  soln and mix. Partially cover vessel with watch-glass and add 10 ml of  $HNO_3$ . Place on steam bath and keep covered until spattering ceases; remove watch-glass and allow evaporation to proceed to dryness, taking care to prevent access of reducing gases or vapors to mixture. Treat residue with 5 ml of 7%  $KMnO_4$  soln and 5 ml of  $HNO_3$  and again evaporate to dryness. Add ca 50 ml of  $H_2O$ , 5 ml of  $HNO_3$ , and 40 ml of saturated  $SO_2$  soln and let stand with occasional stirring (breaking up lumps with glass rod) until hydrated oxide of Mn has dissolved.

Filter, wash paper thoroly with  $H_2O$ , add excess of 10%  $AgNO_3$  soln to combined filtrate and washings, and boil until  $SO_2$  has been expelled and the  $AgI$  has flocculated. Collect precipitate on weighed Gooch crucible, wash first with  $HNO_3$  (1+99), then twice with  $H_2O$ , dry, and weigh. Calculate as percentage of free I and from result subtract percentage of I found as  $NaI$ , 21.54. This result is the I organically combined.

## TOTAL IODINE IN FLUORESCIN DERIVATIVES (31)

## 21.56

## REAGENTS

(a) *Saturated potassium permanganate soln*.—Dissolve ca 70 g of  $KMnO_4$  in 1 liter of  $H_2O$ .

(b) *Dilute potassium permanganate soln*.—Dissolve 1 g of  $KMnO_4$  in 100 ml of  $H_2O$ . This soln should be freshly prepared.

(c) *Potassium nitrite soln*.—Dissolve 100 g of  $KNO_2$  in 1 liter of  $H_2O$ .

(d) *Sulfamic acid soln*.—Dissolve 100 g of  $NH_2SO_3H$  in 1 liter of  $H_2O$ .

(e) *Starch indicator*.—0.5% soln of soluble starch.

(f) *Standard thiosulfate soln*.—0.1 N or 0.05 N soln, depending upon size of sample taken. Standardize against pure  $KIO_3$ .

## 21.57

## DETERMINATION

Weigh into 500 ml Pyrex beaker sufficient sample to give  $Na_2S_2O_3$  titration of ca 30 ml, dissolve in ca 2 ml of 30%  $NaOH$  soln, dilute to 100 ml, and add a few glass beads and 15 ml of the saturated  $KMnO_4$  soln. Boil 5 min. and remove from heat. When boiling ceases, add carefully 10 ml of  $HNO_3$  and boil 5 min. more. Remove beaker from heat and wash down cover and sides. (Excess  $KMnO_4$  must be present at this point.) Add  $KNO_2$  soln dropwise with swirling until soln begins to clear,

then continue adding cautiously, allowing each drop to react before another is added, until practically all the  $\text{MnO}_2$  is reduced. If few particles of  $\text{MnO}_2$  resist reduction, do not attempt to destroy these, but immediately add the dilute  $\text{KMnO}_4$  soln in 1 ml portions until soln becomes pink. If more than 2 ml of the dilute  $\text{KMnO}_4$  soln is required, or if brown color appears, add at once 10 ml of the dilute  $\text{KMnO}_4$  soln, heat to boiling, and repeat dropwise addition of  $\text{KNO}_2$  soln and addition of the dilute  $\text{KMnO}_4$  soln to pink color.

Filter soln rapidly with suction thru sintered glass filter (medium porosity) into wide-mouthed 500 ml flask. Wash beaker and filter thoroly. (Filtrate must be pink.) Add  $\text{KNO}_2$  soln dropwise with shaking until 1 drop has been added in excess of that required to decolorize soln. Add 5 ml of the  $\text{NH}_2\text{SO}_3\text{H}$  soln, wash down sides of flask, and swirl contents until evolution of gas ceases. Cool to room temp., add 2–3 g of KI, and titrate with standard  $\text{Na}_2\text{S}_2\text{O}_3$  soln, using starch indicator. Run blank determinations on reagents used. 1 ml of 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3 = 0.002115$  g of I.

## 21.58

## TOTAL HALOGENS

*Erythrosine*.—Mix 0.5–1 g of dye with 4 g of  $\text{K}_2\text{CO}_3$  and moisten to paste with 50% alcohol. Dry, cover with layer of dry  $\text{K}_2\text{CO}_3$ , and ignite at low red heat. Allow to cool, moisten with few drops of  $\text{H}_2\text{O}$ , and break up charred mass thoroly. Wash into beaker with ca 20 ml of  $\text{H}_2\text{O}$ , allow to digest 15 min., and filter. Wash insoluble matter until washings no longer react with  $\text{AgNO}_3$ ; then acidify filtrate and washings with  $\text{HNO}_3$ , using an excess equivalent to 5 ml of the conc. acid, and precipitate halogens with 10%  $\text{AgNO}_3$  soln. Collect precipitate on weighed Gooch crucible, wash, dry, and weigh. Compare with sum of results obtained in separate halogen determinations.

## 21.59

## SODIUM CARBONATE

*Erythrosine*.—Determine total  $\text{CO}_2$  as directed under 17.6, using 10 g sample. Calculate and report as  $\text{Na}_2\text{CO}_3$ .

## 21.60

## ORANGE II IN ORANGE I

To a volume of  $\text{H}_2\text{O}$  soln of sample that contains 1 g of dye add  $\text{H}_2\text{O}$ , if necessary, to bring volume to 100 ml, and then add 10 ml of  $\text{HCl}$ . Extract this soln by shaking successively in three 500 ml separators, each containing 100 ml of amyl alcohol and 5 ml of  $\text{HCl}$ . Wash each of 3 amyl alcohol extracts by means of six 100 ml portions of N  $\text{Na}_2\text{CO}_3$  soln (53 g of anhydrous  $\text{Na}_2\text{CO}_3$ /liter), passed successively thru separators in order first used. In washing the acidified amyl alcohol solns, shake gently at first, keeping separator upright and unstoppered until evolution of  $\text{CO}_2$  is slow enough to permit more vigorous shaking. In same manner wash extracts in second and third separators with 2 more 100 ml portions of the  $\text{Na}_2\text{CO}_3$  soln and wash extract in third separator with 2 additional portions of the  $\text{Na}_2\text{CO}_3$  soln. Dilute the amyl alcohol solns by adding 350 ml of gasoline (b.p. 90–120°) to each separator. Remove dye by extracting completely with requisite number of 10 ml portions of  $\text{H}_2\text{O}$  passed thru separators, reversing order previously used. Bring volume to 100 or 150 ml by adding  $\text{H}_2\text{O}$ ; add ca 10 g of Na acid tartrate and titrate with standard  $\text{TiCl}_3$  soln, 21.36. 1 ml of 0.1 N  $\text{TiCl}_3 = 0.008756$  g of orange II.

## 21.61

## MARTIUS YELLOW IN NAPHTHOL YELLOW S AND ITS K SALT

Dissolve 5 g of dye in 150 ml of  $\text{H}_2\text{O}$  (500 ml for the K salt), add 5 ml of  $\text{HCl}$ , and shake vigorously in separator 1 min. with 50 ml of gasoline (b.p. 90–120°). Separate solns and extract aqueous liquid again with 25–30 ml of the solvent. Com-

bine portions of gasoline, decant into clean separator, and wash with four 25 ml portions of 0.25 *N* HCl. Remove martius yellow by shaking with few portions of 5% NaOH soln. Neutralize alkaline dye soln with tartaric acid, add Na tartrate, if necessary, and titrate against standard  $\text{TiCl}_3$  soln as directed under 21.39(c). 1 ml of 0.1 *N*  $\text{TiCl}_3$  = 0.002134 g of martius yellow.

Very small quantities (less than 0.1%) may also be determined colorimetrically (in neutral or slightly alkaline soln) by comparison with a standard naphthol yellow S soln, the tinctorial power of which is considered to be 8/10 that of martius yellow.

#### ALIZARIN IN MADDER LAKE—OFFICIAL (32)

##### 21.62

##### REAGENT

*Sodium hydroxide-alcohol soln.*—Mix 60 ml of 10% NaOH soln with 45 ml of alcohol and dilute to 300 ml with  $\text{H}_2\text{O}$ .

##### 21.63

##### DETERMINATION

Weigh 0.2 g of madder lake into 250 ml flask and add 25 ml of 6 *N* HCl and 25 ml of alcohol. Reflux on hot plate 45 min. Cool, and wash all of mixture into 250 ml separator with  $\text{H}_2\text{O}$ . Extract with 50 ml portions of ether until aqueous layer is colorless. Wash combined ether solns with 20 ml portions of the NaOH-alcohol reagent until ether layer is colorless. Combine alkaline solns, acidify with few ml of 6 *N*  $\text{H}_2\text{SO}_4$  (color changes from purple to orange), and re-extract alizarin with six 25 ml portions of ether. Wash combined ether extracts with 25 ml portions of the NaOH-alcohol reagent until ether layer is colorless. Acidify combined washings with a few ml of 6 *N*  $\text{H}_2\text{SO}_4$  and re-extract alizarin with six 25 ml portions of ether. Combine ether extracts and wash 4 times with 25 ml of  $\text{H}_2\text{O}$ . Discard washings. Filter ether soln thru qualitative paper into tared 250 ml crystallizing dish, wash paper with several 20 ml portions of ether, evaporate filtrate at room temp., dry over  $\text{H}_2\text{SO}_4$ , and weigh. Wt. of residue  $\times 500$  = percent alizarin.

#### FD&C RED NO. 4 (PONCEAU SX) IN PRESENCE OF FD&C RED NO. 1 (PONCEAU 3R)

##### 21.64

##### TOTAL COLOR TITRATION

Prepare 1% soln of the dye mixture, and titrate 20 ml portions exactly with 0.1 *N*  $\text{TiCl}_3$ , using Na citrate as a buffer, 21.39(a). In case of over-titration, back-titrate immediately with standard soln of amaranth or other dye.

##### 21.65

##### TITRATION OF FD&C RED NO. 4

Mark a number of 300 ml Erlenmeyer flasks with a suitable agent (crayon, pencil, or glass cutter) at a volume of 40 ml, pipet into marked flasks a quantity of the dye soln equivalent to 12–15 ml of the  $\text{TiCl}_3$  soln, dilute with sufficient  $\text{H}_2\text{O}$  to make 70 ml, and add in the order named 10-ml of 3%  $\text{H}_2\text{O}_2$  (assayed with  $\text{KMnO}_4$  according to U.S.P. method), and 10 ml of 10% NaOH soln. (Total volume in flask should measure finally 90 ml.) Cover flask with short-stemmed funnel and boil briskly over asbestos until 40 ml mark is reached. So control boiling operations that required volume is obtained in ca 2 hours. (In absence of FD&C Red No. 4, resultant soln will be colorless or slightly yellow.)

Permit flask to cool and add ca 100 ml of  $\text{H}_2\text{O}$ , 2.0 g of tartaric acid, and ca 15 g of Na bitartrate. Titrate exactly with  $\text{TiCl}_3$  soln as directed in 21.39(b).

Calculate percentages of FD&C Red No. 4 (Ponceau SX) and No. 1 (Ponceau 3R) from following formulas:

$V_i$  = ml of 0.1 *N*  $\text{TiCl}_3$  before treatment;

$V_o$  = ml of 0.1 *N*  $\text{TiCl}_3$  after treatment;

Weight of FD&C Red No. 4 =  $0.01201 (1.098 V_o - 0.15)$ ; and

Weight of FD&C Red No. 1 =  $0.01236 (V_i - (1.098 V_o - 0.15))$ .

#### TARTRAZINE AND AMARANTH (35)

21.66

##### REAGENTS

(a) *Stannous chloride soln.*—40%. Add 40 g of  $\text{SnCl}_2$  to sufficient  $\text{HCl}$  to make 100 ml.

(b) *Ammonia sodium chloride soln.*—To 12.5 g of  $\text{NaCl}$  and 20 ml of  $\text{NH}_4\text{OH}$  add sufficient  $\text{H}_2\text{O}$  to make 500 ml.

(c) *Starch iodide paper.*—Triturate 10 parts of starch and 200 parts of  $\text{H}_2\text{O}$ , bring to a boil, and add 1 part of  $\text{KI}$ . Impregnate strips of white filter paper with this soln, dry, and preserve in glass-stoppered bottles.

21.67

##### DETERMINATION

Prepare 1% soln of the mixed dyes. Determine total color as directed in 21.39 by titrating definite volumes with 0.1 *N*  $\text{TiCl}_3$ , using  $\text{Na}$  citrate as buffer. A convenient charge (ca 0.2 g of color) requires ca 10 ml of the standard  $\text{TiCl}_3$ , 21.36. Make determinations in duplicate.

Pipet definite volume (20 ml if product is pure color) of the dye soln into 250 ml centrifuge bottles and adjust to volume of 50 ml with  $\text{H}_2\text{O}$ . Add exactly 4 ml of the  $\text{SnCl}_2$  soln, mix well, and permit to stand, preferably overnight at room temp. (not below  $20^\circ$ ). The following day place bottles in water bath previously heated to  $50$ – $60^\circ$ , and maintain contents of bottles at that temp. 5 min. (Since reduction operation has important bearing upon results, above directions must be observed closely.) Remove bottles from bath and cool to room temp. (Reduced solns should be colorless or very faint yellow.) Add exactly 5 ml of  $\text{NH}_4\text{OH}$  and mix with contents, which should become slightly alkaline to litmus paper. Centrifuge, and decant thru 15 cm quantitative filter into 400 ml beaker surrounded by ice  $\text{H}_2\text{O}$ . Into beaker measure 15 ml of  $\text{HCl}$  and 0.2 ml of 10%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  soln. Wash residue in bottles thrice with 50 ml portions of the  $\text{NH}_4\text{OH}$ - $\text{NaCl}$  soln, mix well, centrifuge each time, and decant thru filter. (Total filtrate in beaker should now measure ca 200 ml.) Cool contents of beaker to  $5^\circ$  and add slowly 1 ml of 10%  $\text{NaNO}_2$  soln. Keep temp. between  $5$  and  $8^\circ$  for 2 hours, testing with the starch iodide paper at intervals of ca every 30 min. (There should be an excess of nitrous acid at end of this period. Under ordinary conditions quantity of  $\text{NaNO}_2$  stated is found sufficient.) Add 12 ml of 1% dilute alcoholic  $\beta$ -naphthol soln to 100 ml of 2 *N*  $\text{Na}_2\text{CO}_3$  in liter beaker and cool to  $15^\circ$ . Into  $\beta$ -naphthol soln pour gradually the diazo soln, stirring vigorously. Rinse beaker with some of dye soln and lastly rinse with 25 ml of alcohol and add to dye soln. Heat contents of beaker over steam bath, maintaining temp. of ca  $70^\circ$  for 1 hour, cool, and allow to stand overnight at room temp.

Use six separators of 250 ml capacity. Measure into each 50 ml of amyl alcohol. Add to first separator 50 ml of alkaline dye soln. Shake vigorously and wait until sharp separation has occurred; draw off lower layer and pass successively thru the other 5 separators, and lastly discard lower layer. Repeat procedure with 50



ml portions until entire dye soln is extracted. Rinse beaker with 5–50 ml portions of 0.25 *N* HCl, passing them individually thru amyl alcohol extracts, and later discarding. Dilute each amyl alcohol extract with 100 ml of petroleum benzine and extract orange dye with 100 ml portions of 1/128 *N* HCl, shaking vigorously and passing lower extracts thru entire series of separators. Collect extracts containing orange II in liter casserole. Continue washing amyl alcohol until all orange color is removed. (It will be noted that first separator will readily give up orange dye inasmuch as the red is not readily washed out at that acid concentration.) Continue acid extraction until each separator will yield no more orange color.

In presence of large amount of red color, wash last dilute acid extract with mixture of amyl alcohol and petroleum benzine (1+2) in order to remove any red dye that may have been extracted. Add excess of  $\text{NH}_4\text{OH}$  (10 ml) to casserole containing

TABLE 4.—Percentage of sulfur, nitrogen, and sodium in permitted food dyes

DYE	SULFUR	NITROGEN	SODIUM	SODIUM SULFATE CORRESPONDING TO SODIUM CON- TENT
Amaranth.....	15.91	4.64	11.41	35.28
Ponceau 3R.....	12.97	5.66	9.30	28.71
Ponceau SX.....	13.35	5.83	9.57	29.55
Erythrosine.....	.....	.....	5.12	15.81
Orange I.....	9.15	8.00	6.57	20.28
Naphthol yellow S.....	8.95	7.82	12.84	39.64
Naphthol yellow S-K salt.....	8.21	7.17	.....	.....
Sunset yellow FCF.....	14.18	6.19	10.17	31.40
Tartrazine:				
Trisodium salt.....	12.00	10.49	12.91	39.85
Disodium salt.....	12.52	10.94	8.98	27.72
Guinea green B <sup>1</sup> .....	9.29	4.06	3.33	10.28
Light green SF yellowish: <sup>1</sup>				
Disodium salt.....	12.13	3.53	5.80	17.90
Monosodium salt.....	12.48	3.64	2.98	9.20
Fast green FCF.....	11.89	3.46	5.69	17.57
Brilliant blue FCF.....	12.13	3.53	5.80	17.91
Indigotine.....	13.75	6.01	9.86	30.42
Yellow AB.....	.....	17.00	.....	.....
Yellow OB.....	.....	16.09	.....	.....
Orange SS.....	.....	10.69	.....	.....
Oil red XO.....	.....	10.15	.....	.....

<sup>1</sup> There is evidence that the disodium salt of guinea green B and the trisodium salt of light green SF yellowish form colorless solutions.

the orange color, and evaporate carefully to dryness on steam bath, avoiding spattering. Dissolve coloring matter from casserole with four 25 ml portions of hot  $\text{H}_2\text{O}$  and transfer to 300 ml Erlenmeyer flask. Rinse casserole with 10 ml of alcohol and add to flask; then add 10 g of Na bitartrate and also 1 drop of 2% light green SF yellowish soln (indicator). Boil vigorously, pass in rapid stream of  $\text{CO}_2$ , and titrate with standard  $\text{TiCl}_3$  soln, 21.36, until green color is visible. Note reading and add another drop of standard soln, whereupon green color should be destroyed.

The number of ml of standard  $\text{TiCl}_3$  soln required to reduce the orange II corresponds to quantity of tartrazine originally present. 1 ml of 0.1 *N*  $\text{TiCl}_3$  = 0.01336 g of tartrazine.

Subtract above titration from original total color titer. Difference is volume nec-

essary to reduce amaranth originally present. 1 ml of 0.1 N  $\text{TiCl}_3 = 0.01511$  g of amaranth.

## 21.68

## TOTAL NITROGEN

(a) *Guinea green B, light green SF yellowish, fast green FCF, brilliant blue FCF, and indigotine.*—Proceed as directed under 2.25, using 2 g of sample and a little  $\text{CuSO}_4$  to assist oxidation.

(b) *Amaranth, ponceau 3R, ponceau SX, orange I, sunset yellow FCF, tartrazine, yellow AB, yellow OB, naphthol yellow S and its K salt, orange SS, and oil red XO.*—Treat 2 g of the dye with 25 ml of saturated  $\text{SO}_2$  soln and 1 g of Zn dust (for oil-soluble dyes add a few ml of alcohol) and warm mixture gently until colorless (2–3 min.); if it does not become colorless, add more  $\text{SO}_2$  soln in small portions at a time until color is destroyed. Then add 30 ml of  $\text{H}_2\text{SO}_4$  and 0.7 g of  $\text{HgO}$  or its equivalent of metallic Hg and digest mixture. Finally make alkaline, distil, and titrate as directed under 2.24, 2.25, or 2.26.

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## 22. DAIRY PRODUCTS

### MILK

#### 22.1

##### COLLECTION OF SAMPLE—OFFICIAL

Quantity of sample required depends upon number of determinations to be made. For usual analysis collect 250–500 ml ( $\frac{1}{2}$ –1 pint) of sample; for fat determination only, 50–60 ml (ca 2 fl. oz.) will suffice.

For bottled milk collect one or more bottles as prepared for sale. Thoroughly mix bulk milk by pouring from one clean vessel into another 3 or 4 times or stir at least 30 seconds with utensil reaching to bottom of container. If cream has formed, detach all of it from sides of vessel and stir until liquid is evenly emulsified.

Place in non-absorbent, air-tight containers and keep cold, but above freezing temp., until examined. When transporting samples by mail, express, or otherwise, completely fill containers, tightly stopper, and mark for identification. Suitable quantity of preservative ( $\text{HgCl}_2$ ,  $\text{K}_2\text{Cr}_2\text{O}_7$ , or  $\text{HCHO}$ ) may be used unless presence of preservative is objectionable in connection with physical or chemical tests to be applied in addition to determination of fat.

#### 22.2

##### PREPARATION OF SAMPLE—OFFICIAL

Before withdrawing portions for analytical determinations, bring sample to 15–20° and mix thoroughly by pouring into clean receptacle and back until homogeneous mixture is assured. If lumps of cream do not completely disappear, warm sample to ca 38°, mix thoroughly, then cool to 15–20°. In case a measured volume is required in a determination, bring temp. of sample to 20° before pipetting.

#### 22.3

##### SPECIFIC GRAVITY—TENTATIVE

Determine sp. gr. at 15.6/15.6° with pycnometer or standard hydrometer.

#### 22.4

##### ACIDITY—TENTATIVE

Dilute 10–20 ml of milk with equal volume of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  and titrate with standard  $\text{NaOH}$ , using phenolphthalein indicator. Express result as percentage of lactic acid. Determination is conveniently made by measuring 17.6 ml of prepared sample with Babcock pipet, 22.26(b), diluting with equal volume of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ , which also rinses pipet, and titrating with 0.1  $N$   $\text{NaOH}$ , using 0.5 ml of 1% phenolphthalein soln. Number of ml of 0.1  $N$   $\text{NaOH}$  required  $\div 20$  = percent acid as lactic acid.

##### CITRIC ACID (1)—TENTATIVE

#### 22.5

##### PREPARATION OF SAMPLE

To 50 g of milk in 150 ml beaker, add ca 100 mg of tartaric acid and 6 ml of normal  $\text{H}_2\text{SO}_4$  and heat on steam bath 15 min. Immediately add 3 ml of 20% phosphotungstic acid soln, mix well, and return to steam bath for 5 min. Transfer to 250 ml volumetric flask with alcohol, cool, dilute to mark with alcohol, mix, and filter thru folded paper. Pipet 200 ml of clear filtrate into centrifuge bottle.

#### 22.6

##### REAGENTS

Use reagents specified under 26.36.

## 22.7

## DETERMINATION

To soln in centrifuge bottle, add 10 ml of the Pb acetate soln, shake vigorously ca 2 min., and centrifuge at ca 1000 r.p.m. for 15 min. Carefully decant supernatant liquid from precipitated Pb salts and test with small quantity of the Pb soln. If precipitate forms, return to centrifuge bottle, add more Pb soln, shake, and again centrifuge. If sediment lifts, repeat centrifuging, increasing speed and time. Allow bottle to drain thoroly by inverting several minutes. To the Pb salts in centrifuge bottle add ca 150 ml of H<sub>2</sub>O, shake thoroly, and pass in H<sub>2</sub>S to saturation. Transfer to 250 ml flask, dilute with H<sub>2</sub>O to mark, mix, and filter thru folded paper.

From this point proceed as directed under 26.37, beginning "Pipet 200 ml of filtrate into 500 ml Erlenmeyer flask and evaporate to 75 ml."

Calculate mg of citric acid in portion taken for analysis by following formula:  $X = 0.695P + 0.028S$ , in which  $X$  = mg of citric acid in portion taken for analysis;  $P$  = weight of pentabromacetone (mg); and  $S$  = volume of filtrate (ml).

## LACTIC ACID (S)—OFFICIAL

## 22.8

## PREPARATION OF SOLUTION

(a) *Liquid, whole, and skim milks.*—Weigh 50 g into 100 ml volumetric flask.

(b) *Dried, whole, and skim milks.*—Weigh 5 g into 100 ml beaker and, with heavy stirring rod, make into smooth paste with H<sub>2</sub>O. Transfer contents of beaker to 100 ml volumetric flask with ca 50 ml of H<sub>2</sub>O.

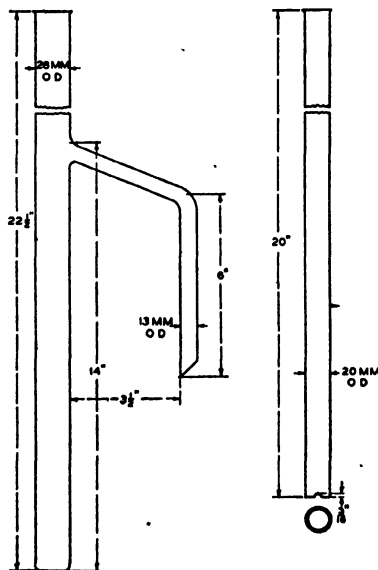


FIG. 30. LIQUID EXTRACTOR

(c) *Cream and ice cream.*—Weigh 20 g into 100 ml volumetric flask and add ca 50 ml of H<sub>2</sub>O.

(d) *Sweetened condensed milk.*—Weigh 25 g into 100 ml beaker and transfer to 100 ml volumetric flask with ca 50 ml of H<sub>2</sub>O.

(e) *Evaporated milk*.—Weigh 25 g into 100 ml volumetric flask and add ca 50 ml of  $H_2O$ .

To the mixtures add 6 ml of normal  $H_2SO_4$  and mix, avoiding vigorous agitation. Add 5 ml of 20% phosphotungstic acid soln (1 ml in the case of cream and 2 ml in the case of ice cream) and make to mark with  $H_2O$ . Shake, and filter thru folded filter paper.

(f) *Butter*.—Weigh 20 g into centrifuge bottle, add 25 ml of  $H_2O$  and warm on steam bath. Neutralize contents of bottle with normal NaOH, using phenolphthalein as indicator. Cool, add 50 ml of ether, and mix well, avoiding violent agitation. Add 50 ml of petroleum benzine, mix well, and centrifuge. Draw off ether layer as completely as possible by means of siphon having lower end bent upward. Repeat extraction, using 25 ml of each of the ethers. Place bottle on steam bath in order to remove most of remaining ether. Transfer residue in bottle to 100 ml volumetric flask, add 3 ml of normal  $H_2SO_4$ , and mix. Cool mixture and precipitate proteins with 20% phosphotungstic acid soln, adding reagent dropwise until no further precipitation occurs. Make to mark, shake, and filter thru a folded filter paper.

## 22.9

## REAGENTS

(a) *Standard barium lactate soln*.—Dissolve in ca 10 ml of  $H_2O$  a quantity of a pure lactic acid salt, such as Li, Zn, or Ca lactate, that will contain equivalent of ca 300 mg of free lactic acid. Transfer material to extractor (Fig. 30), add 0.5 ml of  $H_2SO_4$  (1+1), and adjust volume to 50 ml. Extract with ether for 3 hours. Add ca 20 ml of  $H_2O$  to extraction flask, evaporate ether on steam bath, and carefully titrate contents of flask with 0.1 N  $Ba(OH)_2$ . Transfer neutralized material to 200 ml volumetric flask, make to mark, and shake. Pipet into 500 ml volumetric flask such a quantity of this barium lactate soln as will contain the equivalent of exactly 250 mg of free lactic acid, make to mark, shake, and designate as *standard lactate soln*. (2 ml of this soln will contain the equivalent of 1 mg of lactic acid. For plotting the standard curve this soln must be freshly prepared and promptly used.) Transfer 20 ml of the standard lactate soln to 100 ml volumetric flask, make to mark, and designate as *dilute standard lactate soln*. (10 ml of this soln will contain the equivalent of 1 mg of lactic acid.)

(b) *Carbon*.—To 10 g of high-grade carbon (Nuchar W is suitable, and Suchar, Darco G60, and Carbox E can also be used) in 600 ml beaker, add ca 200 ml of  $H_2O$  and 30 ml of normal HCl, and place on steam bath for 20 min. Agitate continuously with air passed thru cotton. Filter on Büchner funnel and suck as dry as possible, tamping with flattened rod. Transfer cake to beaker, add ca 200 ml of  $H_2O$ , mix thoroly, and refilter. Repeat washing and filtering twice and dry in water oven.

(c) *Ferric chloride soln*.—Dissolve 2 g of  $FeCl_3 \cdot 6H_2O$  in  $H_2O$ , add 5 ml of normal HCl, and dilute to 200 ml.

## 22.10

## PLOTTING STANDARD CURVE

Transfer from buret to volumetric flasks graduated at 50 and 55 ml the quantities of standard solns listed in left-hand column of 22.11. In the right-hand column are given the quantities of lactic acid (mg) that will be contained in the 40 ml of filtrate obtained from each sample after the C treatment described below, and that will therefore be read in the photometer. A blank using 40 ml of  $H_2O$  in place of the designated quantities of lactate soln must be included in the series.

To each flask add 6.6 ml of 0.1 N HCl and  $H_2O$  until volume is ca 40 ml. Now add 200 mg ( $\pm 1$  mg) of the prepared C, shake, and place on steam bath for 10 min.,

mixing at frequent intervals. Cool, make to 55 ml mark with  $H_2O$  at room temp., and as soon as possible filter thru quantitative paper, pouring back until bright.

Transfer 40 ml of each clear filtrate to 50 ml Nessler tube. As the 40 ml of filtrate used contains only 4.8 ml of the acid added during the C treatment, add 1.2 ml of 0.1  $N$   $HCl$ . (A total of 6 ml of 0.1  $N$   $HCl$  is required in the tube.) Place each tube in a jacket of black paper. With one tube at a time, add 5 ml of the  $FeCl_3$  soln by pipet, make to mark, and mix. Pour soln into 4" photometer cell (preferably Pyrex) with plane parallel fused ends, side walls of which are painted black, and read in neutral wedge photometer, using filter No. 46. (Other types of photometers can be used. On exposure to direct light the color fades, but protected as provided it is found to be stable a number of hours.) From the readings obtained prepare standard curve, plotting mg of lactic acid as abscissae and scale readings as ordinates. (Large-scale graph paper is recommended in order that more accurate interpolations may be made.)

It is not necessary to prepare a new standard curve when a new batch of C or soln of  $FeCl_3$  is used. However, a blank determination with  $H_2O$  should be made, and if this blank does not coincide with original blank, readings can be brought into conformity with the curve by simply adding to or subtracting from readings observed amount of variance of new blank from old blank.

## 22.11

*Preparation of dilutions for plotting standard curve*

SOLN TO BE TRANSFERRED TO 50-55 ML VOLUMETRIC FLASK	LACTIC ACID IN 40 ML ALIQVOT
Dilute standard lactate soln	
ml	mg
6.90	0.5
13.80	1.0
27.60	2.0
Standard lactate soln	
8.25	3.0
11.00	4.0
13.75	5.0
16.50	6.0
19.25	7.0
22.00	8.0
24.75	9.0
27.50	10.0
30.25	11.0
33.00	12.0

## 22.12

## EXTRACTION

Place 50 ml of the filtrate obtained from prepared sample and 0.5 ml of  $H_2SO_4$  (1+1) in inner tube of extractor and connect to longest bulb-type condenser available, having an outlet not less than  $\frac{1}{2}$ " inside diam. to obviate regurgitation of the ether. Run  $H_2O$  thru condenser in sufficient quantity to obtain maximum condensation efficiency. Connect extraction flask containing 200 ml of ether, and lower flask onto hot plate that has been previously heated in order to prevent super-heating of the ether. Protect extractor from heat of hot plate by upright sheet of asbestos and extract until all lactic acid is extracted. When ether in extraction flask is kept at rapid boiling and condensing  $H_2O$  is sufficiently cold to allow condensed ether to return to extraction flask in steady stream, a 3-hour extraction period will deliver all the lactic acid. When this rate of extraction cannot be maintained because of

high temp. of the  $H_2O$  passing thru condenser, continue extraction until equivalent of 7500 ml of ether has passed thru soln being extracted. The time required, "T," established for each set of new conditions, is calculated from 2 factors: "A," quantity of ether necessary to fill extractor to overflowing at side-arm, which is a constant for each apparatus; and "B," time in minutes required for quantity "A" to pass from extraction flask and to fill extractor. To determine "A," place 50 ml of  $H_2O$  and 0.5 ml of  $H_2SO_4$  (1+1) in extractor. With extractor held upright, carefully pour ether into inner tube until it just starts passing out of side-arm. Determine "B" in ordinary course of starting each determination. With stop-watch, record interval from time ether first drops from condenser and falls into inner tube to time first drops return to extraction flask from overflow into side-arm. The time, "T", necessary for 7500 ml to pass thru apparatus equals  $7500 \text{ "B"/"A."}$  The calculated "T" holds true only if the rate of boiling and condensing is unchanged thruout extraction period.

### 22.13

#### DETERMINATION

To flask containing ether extract add 20 ml of  $H_2O$  and expel ether on steam bath. Do not allow flask to remain on steam bath after ether has been expelled. Neutralize contents of flask with saturated  $Ba(OH)_2$  soln (phenolphthalein indicator). Wash into 110 ml volumetric flask with alcohol until volume is ca 90 ml. Heat almost to boiling on steam bath, cool, make to mark with alcohol, and filter thru quantitative paper. To expel alcohol, evaporate 100 ml of filtrate to ca 10 ml, add ca 50 ml of  $H_2O$ , and again evaporate to ca 10 ml (or the 100 ml of filtrate may be evaporated to dryness on steam bath). Add from buret 6.6 ml of 0.1 N HCl and transfer contents of beaker with  $H_2O$  to 50–55 ml volumetric flask until volume is ca 40 ml. Add 200 mg of the prepared C, immediately shake, and place on steam bath for 10 min., mixing at frequent intervals. Cool, make to mark with  $H_2O$  at room temp., and filter thru quantitative paper, pouring back until bright.

Transfer 40 ml of filtrate to Nessler tube. (A total of 6 ml of 0.1 N HCl is required in tube. The 40 ml of filtrate contains 4.8 ml of 0.1 N HCl added during the C treatment.) To provide the 6 ml of 0.1 N HCl required, add 1.2 ml of the acid. Place tube in jacket of black paper, add from buret or pipet 5 ml of the  $FeCl_3$  soln, make to 50 ml mark, and mix. (After color has been developed, diluting for purpose of reducing color intensity is not permissible.) Fill a 4" cell, walls of which are painted black, with the soln and read in photometer, using filter No. 46. (Use the same cell, photometer, and color filter used in obtaining standard curve. If photometer is not available, comparisons can be made in conventional manner with Nessler tubes.) Estimate quantity of lactic acid present in the 40 ml portion taken, from standard curve of instrument. If the quantity of lactic acid in 40 ml portion of filtrate exceeds the 12 mg limit of the standard curve, repeat estimation on 10 ml portion of remaining filtrate. The 10 ml portion will contain 1.2 ml of 0.1 N HCl and 4.8 ml. of the acid will have to be added to complete the 6 ml required in Nessler tube. Report lactic acid in terms of mg/100 g.

#### TOTAL SOLIDS

### 22.14

#### Method I.—Official

Weigh 2.5–3 g of prepared sample, 22.2, into weighed flat-bottomed dish not less than 5 cm in diam., using nearer 5 g and a Pt dish if ash is to be determined on same portion. Heat on steam bath 10–15 min., exposing maximum surface of dish bottom to live steam, then heat for 3 hours in air oven at 98–100°. Cool in desiccator, weigh quickly, and report per cent residue as total solids.



## 22.15

*Method—(Approximate).—Tentative*

Determine sp. gr. of milk with Quevenne lactometer (take reading at top of meniscus), observe temp., and correct reading  $L$  to 60° F. by 44.26. Calculate total solids either from formula  $0.25 L + 1.2 F$ , in which  $F$  = percentage of fat in milk, or from 44.27.

## 22.16

## ASH—TENTATIVE

Into suitable Pt dish weigh ca 10 g of prepared sample, 22.2, and evaporate to dryness on steam bath. Ignite in muffle at temp. not higher than 550° until ash is free from C. Cool in desiccator, weigh, and calculate per cent ash.

## 22.17

## TOTAL NITROGEN—OFFICIAL

Transfer 5 g of sample to Kjeldahl digestion flask and proceed as directed under 2.24, 2.25, or 2.26. Percentage of N  $\times 6.38$  = percentage of "protein."

## CASEIN

(Determination should be made while milk is fresh or nearly so. If not possible to make determination within 24 hours, add 1 part of HCHO to 2500 parts of milk and keep in cool place.)

## 22.18

*Method I.—Official*

Place 10 g of sample in beaker with 90 ml of H<sub>2</sub>O at 40–42° and add at once 1.5 ml of acetic acid (1+9). Stir, and let stand 3–5 min. Decant on acid-washed filter, wash by decantation 2 or 3 times with cold H<sub>2</sub>O, and transfer precipitate to filter. Wash once or twice on filter. (Filtrate should be clear, or nearly so.) If first portions of filtrate are not clear, repeat filtration, and complete washing of precipitate. Determine N in washed precipitate and filter paper as directed under 2.24, 2.25, or 2.26, and multiply by 6.38 to obtain equivalent of casein.

To sample of milk that has been preserved the acetic acid should be added a few drops at a time with stirring, and addition should be continued until liquid above precipitate becomes clear, or very nearly so.

*Method II (3)—Tentative*

## 22.19

## REAGENT

Pipet 250 ml of normal acetic acid into 1000 ml flask. Add 125 ml of normal CO<sub>2</sub>-free NaOH. Make up to 1000 ml with CO<sub>2</sub>-free H<sub>2</sub>O and mix thoroly.

## 22.20

## DETERMINATION

Pipet 20 ml of sample into 100 ml flask. Add 50 ml of the reagent, mix, make up to volume with H<sub>2</sub>O, and shake well. Set flask in hot H<sub>2</sub>O (50–60°, *not over 60°*) and let stand 15 min. Cool to room temp., add 0.5 g of celite analytical filter aid, shake thoroly, and filter clear thru suitable folded paper, taking care to prevent evaporation during filtration. Determine N (A) in 50 ml of the clear filtrate, and determine total N (B) in 10 ml of the milk.  $(B - A) \times 6.38$  = casein in 10 ml of the milk. Report g of casein/100 ml of milk, or divide g/100 ml by density of milk and report as percentage by weight.

## 22.21

## ALBUMIN—OFFICIAL

Exactly neutralize filtrate obtained under 22.18 with 10% NaOH, add 0.3 ml of acetic acid (1+9), and heat on steam bath until albumin is completely precipitated.

Collect precipitate on acid-washed filter; wash with cold  $H_2O$ ; determine N as directed under 2.24, 2.25, or 2.26; and multiply by 6.38 to obtain equivalent albumin.

### LACTOSE

#### *Polarimetric Method (4)—Official, First Action*

##### 22.22

##### REAGENTS

(a) *Acid mercuric nitrate soln.*—Dissolve Hg in twice its weight of  $HNO_3$  and dilute with a five-fold volume of  $H_2O$ .

(b) *Mercuric iodide soln. (5)*—Dissolve 33.2 g of KI and 13.5 g of  $HgCl_2$  in 200 ml of acetic acid and 640 ml of  $H_2O$ .

##### 22.23

##### DETERMINATION

Weigh 65.8 g (2 N weight) of milk into each of 2 volumetric flasks, 100 ml and 200 ml respectively. Add 20 ml of the acid Hg ( $NO_3$ )<sub>2</sub> soln or 30 ml of the  $HgI_2$  soln to each flask, followed by 15 ml of 5% phosphotungstic acid soln to the 200 ml flask, and sufficient phosphotungstic acid soln to the 100 ml flask to make to mark. Fill 200 ml flask to mark with  $H_2O$ , shake both flasks frequently during 15 min., filter thru dry filter, and polarize. (It is preferable to read soln from 200 ml flask in 400 mm tube to reduce error of reading; soln from 100 ml flask may conveniently be read in 200 ml tube.) Compute per cent lactose in sample as follows: (1) Subtract reading of soln from 200 ml flask (using 400 mm tube) from reading of soln from 100 ml flask (using 200 mm tube); (2) multiply difference by 2; (3) subtract result from reading of soln from 100 ml flask; (4) divide result by 2.

##### 22.24

#### *Gravimetric Method—Official*

Dilute 25 g of sample with 400 ml of  $H_2O$  in 500 ml volumetric flask and add 10 ml of  $CuSO_4$  soln, 34.33(a), and ca 7.5 ml of a KOH soln of such concn. that 1 volume is just sufficient to precipitate completely the Cu as hydroxide from 1 volume of the  $CuSO_4$  soln. (Instead, 8.8 ml of 0.5 N NaOH soln may be used.) After addition of alkali soln, mixture must still have an acid reaction and contain Cu in soln. Fill flask to mark, mix, filter thru dry filter, and determine lactose in aliquot of filtrate as directed under 34.39. Obtain from 44.11, weight of lactose equivalent to weight of  $Cu_2O$ .

### FAT

##### 22.25

#### *Roese-Gottlieb Method (6)—Official*

Transfer 10 g of sample to Mojonnier fat-extraction flask or Röhrig tube. Add 1.25 ml of  $NH_4OH$  (2 ml if sample is sour) and mix thoroly. Add 10 ml of alcohol and mix well. Add 25 ml of ether, stopper with cork or stopper of synthetic rubber unaffected by usual fat solvents, and shake very vigorously 1 min. Add 25 ml of petroleum benzine (redistilled slowly at temp. below 65°) and repeat vigorous shaking. Centrifuge Mojonnier flask 20 min. at ca 600 r.p.m. or let it (or Röhrig tube) stand until upper liquid is practically clear. Decant ether soln into suitable flask or metal dish. Wash lip and stopper of extraction flask or tube with mixture of equal parts of the two solvents; add washings to weighing flask or dish. Twice repeat extraction of liquid remaining in flask or tube, using 15 ml of each solvent each time. Evaporate solvents on hot plate or steam bath at temp. that effects complete evaporation, but not so high that spattering or vigorous bumping will result. Dry fat in oven at temp. of boiling  $H_2O$  to constant weight. Weigh cooled flask or dish,

using as counterpoise a duplicate container handled in same manner, and avoid wiping either immediately before weighing. Remove fat completely from container with warm petroleum benzine, dry, and weigh as before. Deduct from total weight. Loss in weight = weight of fat. Correct weight of fat by blank determination on reagents used.

*Babcock Method (7)—Official*

**22.26**

**APPARATUS**

(a) *Standard Babcock test milk bottle.*—8%, 18-g, 6" milk-test bottle, total height 150–165 mm (5.9–6.5"). Bottom of bottle shall be flat, and axis of neck shall be vertical when bottle stands on level surface. Charge of milk for bottle shall be 18 g.

*Bulb.*—Capacity of bulb to junction with neck shall be not less than 45 ml. Shape of bulb shall be either cylindrical or conical. If cylindrical, outside diameter shall be between 34 and 36 mm; if conical, outside diameter of base shall be between 31 and 33 mm, and maximum diameter between 35 and 37 mm.

*Neck.*—Shall be cylindrical and of uniform diameter from at least 5 mm below lowest graduation mark to at least 5 mm above highest. Top of neck shall be flared to diameter of not less than 10 mm. Graduated portion of neck shall have length of not less than 63.5 mm. Total per cent graduation shall be 8. Graduations shall represent whole per cent, 0.5%, and 0.1%, respectively, from 0.0 to 8.0%. Tenths per cent graduations shall be not less than 3 mm in length; 0.5% graduations shall be not less than 4 mm in length and shall project 1 mm to left; and whole per cent graduations shall extend at least half-way around neck to right and shall project at least 2 mm to left of tenths per cent graduations. Each whole per cent graduation shall be numbered, number being placed to left of scale. Capacity of neck for each whole per cent on scale shall be 0.20 ml. Maximum error of total graduation or any part thereof shall not exceed volume of smallest unit of graduation.

Each bottle shall be so constructed as to withstand stress to which it will be subjected in centrifuge.

(a) *Testing.*—The Hg and cork, alcohol and buret, and alcohol and brass plunger methods may be used for rapid testing of bottles, but accuracy of any questionable bottle shall be determined by calibration with Hg (13.5471 g of clean, dry Hg at 20° to be equal to 5% on the scale of an 18 g bottle and 10% on the scale of a 9 g bottle), bottle being previously filled to zero with Hg.

(b) *Pipet.*—The standard milk pipet shall conform to following specifications:

	mm
<i>Total length, not more than</i> .....	330
<i>Outside diameter of suction tube</i> .....	6–8
<i>Length of suction tube</i> .....	130
<i>Outside diameter of delivery tube</i> .....	4.5–5.5
<i>Length of delivery tube</i> .....	100–120
<i>Distance of graduation mark above bulb</i> .....	15–45
<i>Nozzle, straight</i> .....	
<i>Graduation, to contain 17.6 ml of H<sub>2</sub>O at 20° when bottom of meniscus coincides with mark on suction tube.</i>	
<i>Delivery, 5–8 seconds.</i>	
<i>Maximum error in graduation, not to exceed 0.05 ml.</i>	
<i>Pipet is to be marked "Holds 17.6 ml."</i>	

(b) *Testing.*—Pipet shall be tested by measuring from buret the volume of H<sub>2</sub>O (at 20°) which it holds up to graduation mark.

(c) *Acid measure.*—The device used to measure  $H_2SO_4$ , whether graduated cylinder or pipet attached to Swedish acid bottle, shall be graduated to deliver 17.5 ml.

(d) *Centrifuge or "tester."*—The standard centrifuge, however driven, shall be constructed thruout and so mounted as to be capable, when filled to capacity, of rotating at necessary speed with minimum of vibration and without liability of causing injury or accident. It shall be heated, electrically or otherwise, to temp. of at least  $55^\circ$  during process of centrifuging. It shall be provided with speed indicator, permanently attached, if possible. Proper rate of rotation may be ascertained by reference to table below. By "diameter of wheel" is meant distance between inside bottoms of opposite cups measured thru center of rotation of centrifuge wheel while cups are horizontally extended.

Diameter of wheel, inches.	10	12	14	16	18	20	22	24
No. revolutions per minute.	1074	980	909	848	800	759	724	693

(e) *Dividers or calipers.*—For measuring fat column.

(f) *Water bath for test bottles.*—Provided with thermometer and device for maintaining temp. of  $55\text{--}60^\circ$ .

## 22.27

### DETERMINATION

Transfer 18 g of prepared sample, 22.2, to milk-test bottle by means of pipet. Blow out milk remaining in pipet tip after free outflow has ceased. Add 17.5 ml of  $H_2SO_4$  (sp. gr. 1.82–1.83 at  $20^\circ$ ) in small portions and in such a way as to wash all traces of milk into bulb. Temp. of acid shall be  $15\text{--}20^\circ$ . Shake until all traces of curd have disappeared; then transfer bottle to centrifuge; counter-balance it; and, after proper speed has been attained, whirl 5 min. Add soft  $H_2O$  at  $60^\circ$ , or above, until bulb of bottle is filled. Whirl 2 min. Add hot  $H_2O$  until liquid column approaches top graduation of scale. Whirl 1 min. longer at temp. of  $55\text{--}60^\circ$ . Transfer bottle to warm water bath maintained at temp. of  $55\text{--}60^\circ$ , immerse it to level of top of fat column, and leave until column is in equilibrium and lower fat surface has assumed a final form. Remove bottle from bath, wipe it, and with aid of dividers or calipers measure fat column, in terms of percentage by weight, from its lower surface to highest point of upper meniscus.

The fat column, at time of measurement, should be translucent, of golden-yellow or amber color, and free from visible suspended particles. Reject all tests in which fat column is milky or shows presence of curd or of charred matter, or in which reading is indistinct or uncertain.

### ADDED WATER

## 22.28

### Acetic Serum Method (8)—Official

(a) *Zeiss immersion refractometer reading.*—To 100 ml of the milk, measured at  $20^\circ$  into beaker, add 2 ml of 25% acetic acid (sp. gr. 1.035). Cover beaker with watch-glass, place in water bath at  $70^\circ$  for 20 min., then in ice  $H_2O$  for 10 min., and separate curd from serum by rapid filtration thru small filter. Transfer portion of clear serum to refractometer beaker, place in constant temp. bath, and take refractometer reading when temp. of serum has been brought to exactly  $20^\circ$ , as determined by thermometer graduated in tenths of a degree. Reading below 39 indicates added  $H_2O$ ; between 39 and 40, addition of  $H_2O$  is suspected. When reading is 40 or below, determine ash in serum as directed under (b).

(b) *Ash.*—Transfer 25 ml of serum to weighed flat-bottomed Pt dish and evaporate to dryness on water bath. Heat over low flame (to avoid spattering) until contents are thoroly charred, place dish in muffle, preferably with pyrometer attached, and

ignite to white ash at temp. not greater than 500°. Cool and weigh. Express result as g/100 ml. Result below 0.715 g/100 ml indicates added H<sub>2</sub>O. Acetic serum ash  $\times 1.021$  = sour serum ash (dilution of acetic serum being 2%).

**22.29***Sour Serum Method—Official*

(a) *Zeiss immersion refractometer reading (9).*—Allow milk to become completely sour, filter, and determine immersion refractometer reading of clear serum at 20°. Reading below 38.3 indicates added H<sub>2</sub>O.

(b) *Ash (10).*—Determine ash in 25 ml of serum obtained in (a) as directed under 22.28(b). Result below 0.730 g/100 ml indicates added H<sub>2</sub>O.

**22.30***Copper Serum Method (11)—Official*

To 1 volume of CuSO<sub>4</sub> soln (72.5 g of CuSO<sub>4</sub> · 5H<sub>2</sub>O per liter, adjusted if necessary to read 36 at 20° on scale of Zeiss immersion refractometer, or to sp. gr. of 1.0443 at 20/4°), add 4 volumes of milk. Shake well and filter. Determine refractometer reading of clear serum at 20°. Reading below 36 indicates added H<sub>2</sub>O. When refractometer reading is 36 or below, determine ash of sour serum as directed under 22.29(b) or of acetic serum as directed under 22.28(b).

*Cryoscopic Method (12)—Official***22.31**

## APPARATUS

(a) *Cryoscope.*—A cylindrical-shaped Dewar flask of 1 liter capacity and 28 cm internal depth, surrounded by metal casing, is tightly closed by means of large cork of ca 3 cm thickness. Thru center of cork is tightly fitted medium thin-walled glass or metal tube, 250 mm in length by 33 mm outside diameter. At one side of cork is inserted narrow metal inlet tube, lower end of which is formed into perforated loop near bottom of flask. At opposite side is metal tube of T-shape construction and 6 mm internal diameter, intended to afford escape for vapors, and also for introducing volatile fluid into apparatus. At back portion of cork is fitted control thermometer, bulb of which extends nearly to bottom of flask. The freezing test tube is of thin glass, ca 240 mm in length by 29 mm outside diameter, and fits closely into larger tube, which is sealed into cork. In rubber stopper of freezing tube is fitted the standard thermometer. The length of thermometer permits insertion of bulb nearly to bottom of tube and at same time allows complete exposure of scale above stopper. At right side of thermometer a stirring device made of non-corrodible low conductivity metal is fitted into stopper thru short section of thin-walled

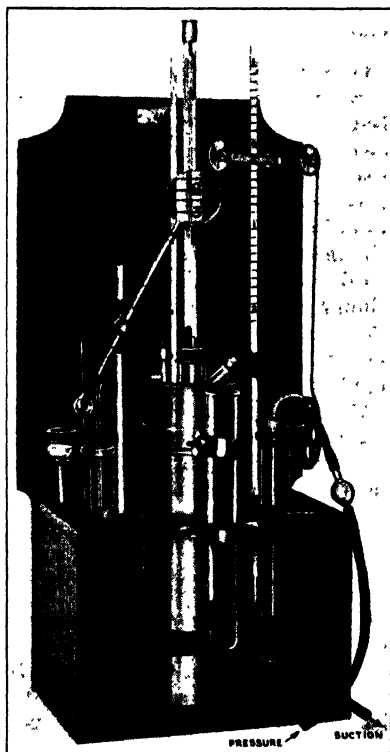


FIG. 31.—HORTVET CRYOSCOPE

metal tubing. The lower end extends nearly to bottom of test tube and is provided with horizontal loop encircling thermometer. At left of thermometer is freezing-starter attachment inserted thru opening in stopper formed by means of short section of metal tubing. This device consists of non-corrodible metal rod, at lower end of which is 10 mm length opening for purpose of carrying small fragment of ice. At one side of cryoscope is installed an air-drying arrangement which consists of a Folin absorption bulb inserted thru tightly fitting stopper and extending nearly to bottom of large-sized test tube. A short section of glass tubing is inserted thru second opening in stopper and is connected to vaporizing tube which enters cryoscope.  $\text{H}_2\text{SO}_4$  is poured into drying tube to level slightly above small inner bulb. At opposite side of apparatus is arranged a drain tube for purpose of conducting vapors away from operator. By means of pressure and suction pump dry air may be forced into apparatus at suitable rate and mixed vapors conducted out thru base of drain tube into sink. An adjustable lens is mounted in convenient position in front of thermometer for purpose of magnifying the scale.

(b) *Standard thermometer*.—A solid-stem instrument having total length of 58 cm, with scale portion measuring ca 30 cm. Total scale range is  $3^\circ$ , from  $+1^\circ$  to  $-2^\circ$ , and each degree division is subdivided into tenths and hundredths. Length of a degree division approximates 1 dm, thus making smallest subdivisions of such magnitudes as to enable easy observation and readings estimated to  $0.001^\circ$ . Standardize thermometer as directed under 22.32. Check at frequent intervals, once a week or as often as may be necessary, to keep accurate record of any changes that may occur.

(c) *Control thermometer*.—Solid-stem instrument ca 58 cm in length and having scale range of  $+20^\circ$  to  $-30^\circ$ . Test in bath of melting crushed ice for purpose of determining whether 0-mark on scale is correct. Scale graduations should be accurate to within  $0.10^\circ$ .

## 22.32

## STANDARDIZATION OF THERMOMETER

Make 3 freezing-point determinations by procedure given under 22.33 on each of following:

(a) *Recently boiled distilled  $\text{H}_2\text{O}$* .

(b) *Sucrose soln.*—Dissolve 7 g of pure sucrose in  $\text{H}_2\text{O}$  and make soln to volume of 100 ml at  $20^\circ$ .

(c) *Sucrose soln.*—Dissolve 10 g of pure sucrose in  $\text{H}_2\text{O}$  and make soln to volume of 100 ml at  $20^\circ$ .

(Pure sucrose may be obtained from Director of National Bureau of Standards, Washington, D. C.)

Tabulate results in following form:

FREEZING- POINT OBSERVATIONS	PURE WATER	7 GRAMS SUCROSE SOLUTION		10 GRAMS SUCROSE SOLUTION	
		Observed freezing point ( $-S$ )	Freezing-point depression $S-W$ (algebraic)	Observed freezing point ( $-S$ )	Freezing-point depression $S-W$ (algebraic)
1st					
2nd					
3rd					
Averages	$\pm W$	xxxxxxx		xxxxxxx	

Express results as degrees freezing-point depression below average of observed freezing points obtained on sample of pure  $\text{H}_2\text{O}$  ( $\pm W$ ), which may be above (+) or below (−) the 0-mark on scale. Obtain each freezing-point depression of the sucrose solns by algebraic subtraction of average of freezing-point readings of pure  $\text{H}_2\text{O}$  ( $\pm W$ ) from each observed freezing point.

Omit adventitious results, *i.e.*, results that are in marked disagreement with other results obtained by carefully following instructions.

Apply average of freezing-point depressions obtained on standard sucrose solns for correcting thermometer readings obtained on sample of milk in manner illustrated in the tables accompanying Fig. 32.

### 22.33

#### DETERMINATION

(Make freezing-point determinations only on samples of milk that show an acidity of not more than 0.18% when determined as directed under 22.4.)

Insert funnel-tube into vertical portion of T-tube at one side of apparatus and pour in 400 ml of ether previously cooled to  $10^\circ$  or lower. Close vertical tube by means of small cork and connect pressure pump to inlet tube of air-drying attachment. Adjust pump so as to pass air thru apparatus at moderate rate, as may be judged by agitation of  $\text{H}_2\text{SO}_4$  in drying tube. Continuous vaporization of ether will cause lowering of temp. in flask from ordinary room temp. to  $0^\circ$  in 5–10 min. Continue temp. lowering until control thermometer registers near  $-3^\circ$ . At this stage, by lowering gage tube into ether bath, then closing top by means of forefinger and raising to suitable height, an estimate can be made as to quantity of ether necessary to pour in for purpose of restoring the 400 ml volume. When volume of ether has been adjusted to 400 ml an additional 10–15 ml is sufficient on an average for each succeeding determination. Pour into freezing test tube sufficient  $\text{H}_2\text{O}$  (30–35 ml), boiled and cooled to  $10^\circ$  or lower, to submerge thermometer bulb. Insert thermometer together with stirrer and lower test tube into larger tube. A small quantity of alcohol, sufficient to fill lower space between the 2 test tubes, will serve to complete conduction medium between freezing bath and liquid to be tested. Keep stirrer in steady up-and-down motion at rate of ca one stroke every 1 or 2 seconds, or even at slower rate, provided cooling proceeds satisfactorily. Maintain passage of air thru apparatus until temp. of cooling-bath reaches  $-2.5^\circ$ , at which time top of Hg thread in thermometer usually recedes to position near freezing point of  $\text{H}_2\text{O}$ . Maintain temp. of cooling-bath at  $-2.5^\circ$  and continue manipulation of stirrer until super-cooling of sample of  $1.0$ – $1.2^\circ$  is observed. As a rule, at this time liquid will begin to freeze, as may be noted by rapid rise of the Hg. Manipulate stirrer slowly and carefully 3 or 4 times as Hg column approaches its highest point. By means of suitable light-weight cork mallet tap upper end of thermometer cautiously a number of times until top of Hg column remains stationary at least 1 min. Observe exact reading on thermometer scale, taking necessary precautions to avoid parallax, and estimate to  $0.001^\circ$ . When observation has been satisfactorily completed, make duplicate determination; then remove thermometer and stirrer and empty  $\text{H}_2\text{O}$  from freezing tube.

Rinse tube with ca 25 ml of sample of milk, cooled to  $10^\circ$  or lower; measure into tube 30–35 ml of milk or enough to submerge thermometer bulb; and insert tube into apparatus. Maintain temp. of cooling-bath at  $2.5^\circ$  below probable freezing point of sample. Make determination on the milk, following same procedure as that used in determining freezing point of  $\text{H}_2\text{O}$ . As a rule, however, it is necessary to start freezing action in milk by inserting the freezing starter (kept in contact with ice for

several minutes, and in open end of which has been wedged a fragment of ice) at time when Hg column has receded to 1.0–1.2° below probable freezing point. A

Two Bureau of Standards tested thermometers gave intervals of 0.199° and 0.200°, respectively, between freezing-point depression readings of the two sucrose solns. One thermometer (Fig. 32) gave freezing-point depressions  $-0.422^{\circ}$  ( $+0.079$  and  $-0.343$ ) and  $-0.621^{\circ}$  ( $+0.079$  and  $-0.542$ ), respectively, for the two sucrose solns, while the other gave  $-0.422^{\circ}$  and  $-0.622^{\circ}$ , respectively.

#### Laboratory Thermometer No. 2.

WATER	7 GRAMS SUCROSE TO 100 ML	10 GRAMS SUCROSE TO 100 ML
Av. $+0.056^{\circ}$	$-0.425^{\circ}$	$-0.621^{\circ}$

Interval = 0.196  
0.196 equiv. 0.199  
Correction factor = 1.015

#### Laboratory Thermometer No. 24.

WATER	7 GRAMS SUCROSE TO 100 ML	10 GRAMS SUCROSE TO 100 ML
Av. $0.000^{\circ}$	$-0.420^{\circ}$	$-0.625^{\circ}$

Interval = 0.205  
0.205 equiv. 0.199  
Correction factor = 0.971

#### Example

Laboratory Thermometer No. 24.  
F. pt. Depression Sample Milk = 0.548  
(0.548  $-$  .420) 0.971 = 0.124  
True freezing point =  $0.422 + 0.124$   
(= 0.546° below zero C)

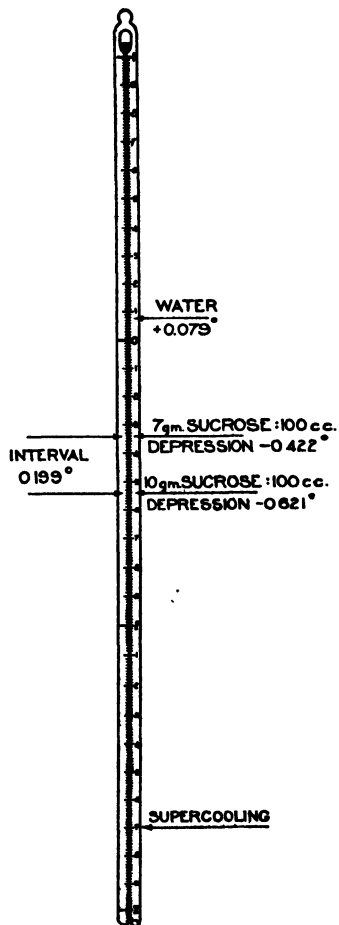


FIG. 32.—U. S. BUREAU OF STANDARDS TESTED THERMOMETER

rapid rise of the Hg results almost immediately. Remove starter and manipulate stirrer slowly and carefully 2 or 3 times while Hg approaches its highest point. Complete adjustment of Hg column in same manner as in preceding determination; then, avoiding parallax, observe exact reading on thermometer scale and estimate to



0.001°. *Algebraic difference* between average of readings obtained on the H<sub>2</sub>O and reading obtained on sample of milk represents *freezing-point depression* of the milk. To determine true *freezing-point* ( $T^1$ ) of milk, subtract from freezing-point depression, freezing-point depression of 7% sucrose soln as determined by the laboratory thermometer. Multiply difference by correction factor for thermometer. Add to product 0.422 (freezing point of 7% sucrose soln by Bur. Standards thermometer). See example under Fig. 32.

Ascertain percentage of added H<sub>2</sub>O corresponding to determined freezing-point depression from 44.28. Percentage of added H<sub>2</sub>O ( $W$ ) may also be calculated as follows:

$$W = \frac{100(T - T^1)}{T}, \text{ in which}$$

$T$  = average freezing point of normal milk ( $-0.550^\circ$ ), and

$T^1$  = true freezing point on given sample.

A tolerance of 3% may be allowed on results for added H<sub>2</sub>O determined on basis of average freezing-point depression of  $-0.550^\circ$ . Owing to narrow variations found in market milks of genuine character it is not necessary to deduct tolerance figure from results showing added H<sub>2</sub>O in excess of 3%.

#### GELATIN (13)

##### 22.34

##### *Qualitative Test—Official*

To 10 ml of the milk add an equal volume of acid Hg(NO<sub>3</sub>)<sub>2</sub> soln (Hg dissolved in twice its weight of HNO<sub>3</sub> and this soln diluted to 25 times its volume with H<sub>2</sub>O), shake mixture, add 20 ml of H<sub>2</sub>O, shake again, allow to stand 5 min., and filter. If much gelatin is present, filtrate will be opalescent and cannot be obtained quite clear. To portion of filtrate in test tube add equal volume of saturated aqueous picric acid soln. Yellow precipitate will be produced in presence of any considerable quantity of gelatin, while smaller quantities will be indicated by cloudiness.

NOTE: In applying this test to sour, fermented, cultured, or very old samples of milk, cream, or buttermilk; to sterilized cream or evaporated milk; or to cottage cheese, use care to recognize precipitates produced by picric acid when added to the Hg(NO<sub>3</sub>)<sub>2</sub> filtrates from these materials in absence of gelatin. Such samples, with or without rennet and entirely free from gelatin, give, on standing, distinct precipitates when treated as above outlined. In every case, however, these precipitates differ in character from those produced by picric acid with gelatin. The gelatin-picric acid precipitate is finely divided, more apt to remain in suspension, settles only slowly, and adheres tenaciously to sides and bottom of container from which it is rinsed with difficulty. Precipitates produced by picric acid in absence of gelatin are flocculent, separate readily (leaving serum practically clear), do not adhere to walls of container, and are easily removed by rinsing with H<sub>2</sub>O. When gelatin is present in sample, the gelatin-picric acid precipitate will remain in suspension long after the flocculent precipitate has settled, but on standing overnight the characteristic sticky deposit will be found adhering tenaciously to bottom and sides of test vessel. If gelatin is present in relatively high concentration (1%), the gelatin-picric acid precipitate will be voluminous and will settle rather quickly.

##### 22.35

##### PRESERVATIVES—OFFICIAL

Proceed as directed under Chap. 32. To test for salicylic acid or benzoic acid acidify 100 ml of the milk with 5 ml of HCl (1+3), shake until curdled, filter, and treat clear filtrate as directed under 32.2, 32.3, or 32.6.

To test for HCHO proceed as directed under 32.20–32.26, inclusive, applying test directly to the milk.

### HYPOCHLORITES AND CHLORAMINES (14)—TENTATIVE

#### 22.36

##### REAGENTS

- (a) *Potassium iodide soln.*—Dissolve 7 g of KI in 100 ml of H<sub>2</sub>O. Prepare fresh.
- (b) *Hydrochloric acid.*—To 100 ml of HCl add 200 ml of H<sub>2</sub>O.
- (c) *Starch soln.*—Boil 1 g of starch in 100 ml of H<sub>2</sub>O. Cool before using.

#### 22.37

##### TESTS

(a) To 5 ml of milk in medium-sized test tube add 1.5 ml of the KI soln, mix thoroly by shaking, and observe color of milk.

(b) If unaltered, add 4 ml of the HCl, mix thoroly by means of glass rod flattened at one end, and note color of curd.

(c) Next place tubes in large H<sub>2</sub>O bath, previously heated to 85°, allow to remain 10 min. (during this interval curd will rise to surface), then cool rapidly by placing in cold H<sub>2</sub>O. Note color of curd and liquid.

(d) Then add 0.5–1 ml of the starch soln to liquid below curd and note color.

#### 22.38

##### *Reactions with the various tests*

CONCENTRATION OF AVAILABLE CL	1:1,000	1:2,000	1 5,000	1:10,000	1:25,000	1:50,000
Test a	Yellowish brown	Deep yellow	Pale yellow, fades	—	—	—
Test b	Yellowish brown	Deep yellow	Light yellow	—	—	—
Test c	Yellowish brown	Deep yellow	Yellow	Yellow	Pale yellow	Yellowish
Test d	Blue purple	Blue purple	Blue purple	Dark red- purple	Red purple	Pale red- purple

#### 22.39

##### COLORING MATTERS (15)—OFFICIAL

Warm ca 150 ml of milk in casserole over flame, add ca 5 ml of acetic acid (1+3), and slowly continue heating nearly to boiling point while stirring. Gather curd, when possible, into one mass with stirring rod and pour off whey. If curd breaks up into small flecks, separate from whey by straining thru sieve or colander. Press curd free from adhering liquid, transfer to small flask, macerate with ca 50 ml of ether, keeping flask tightly corked and shaking at intervals, and allow to stand for several hours, preferably overnight. Decant ether extract into evaporating dish, remove ether by evaporation, and test fatty residue for annatto as directed under 21.16(b).

The curd of an uncolored milk is perfectly white after complete extraction with ether, as is also that of a milk colored with annatto. If extracted fat-free curd is distinctly orange or yellowish in color, a coal tar dye is indicated. In many cases if a lump of fat-free curd in test tube is treated with a little HCl the color changes to pink, indicating presence of dye similar to aniline yellow or butter yellow or perhaps one of the acid azo yellows or oranges. In such cases, separate and identify

coloring matter present in the curd as directed under 21.11. If aniline yellow, butter yellow, or any other oil-soluble dye is present, greater part will be found in ether extract containing the fat. In such cases proceed as directed under 21.3.

In some cases, presence of coal tar dyes can be detected by treating ca 100 ml of milk directly with equal volume of HCl in porcelain casserole, giving dish a slight rotary motion. In presence of some dyes the separated curd acquires a pink coloration.

#### SEDIMENT TEST (16)

(Taken from "Standard Methods of Milk Analysis," American Public Health Association. This method has been edited to conform in part to the style of this publication.)

#### 22.40

##### SAMPLING

Pint samples only are regarded as standard. If quart or any other size of sample is used, report size. Take samples from well-stirred cans or vats of milk or from pint or well-shaken quart bottles of milk. Do not take samples from unstirred bottom milk of 40 quart or other cans. Measure quantity of milk used with reasonable accuracy.

#### 22.41

##### PREPARATION OF SEDIMENT DISKS

Strain the pint sample of milk thru suitable sediment tester fitted with firm cotton disk (type furnished by Lorenz Model Co., Madison, Wis., is suitable) placed over opening 1" in diam. (It will hasten process of filtering if provision is made for warming the milk, or it may be forced thru disk by air pressure.)

#### 22.42

##### PREPARATION OF STANDARD SEDIMENT DISKS

Dry a quantity of weathered cow dung in oven and grind in mill until most of it will pass 100-mesh and practically all will pass 60-mesh screen. Accurately weigh 0.1 g and transfer to 1000 ml flask, using 50% sugar soln to wash all fine particles down into flask. Make volume up to mark with more of the sugar soln after most fine particles have been wetted by shaking the half-filled flask thoroly several times. After volume is made up to mark, shake contents of flask vigorously every 5 min. for sufficient time to saturate particles thoroly ( $\frac{1}{2}$ -1 hr.). When particles have been thoroly wetted it will be noted that the sugar soln will hold them evenly in suspension, and mixture is ready to use in making the standard disks.

On basis of 0.1 g per 1000 ml, 10 ml of the sugar soln contains 1 mg of sediment. Make test disks with one of the usual sediment testers, using varying volumes of the sediment suspension. Place several ounces of filtered skimmed milk in sediment tester and add varying volumes of the sediment suspension. After forcing milk thru disks, run thru small quantity of filtered skimmed milk to obtain a more even distribution of sediment on disk.

Remove disk from tester, mount permanently on stiff paper, allow to dry, and then make permanent by spraying with strong disinfectant such as  $\text{HgCl}_2$ . A good apparatus for this purpose is an ordinary throat atomizer, provided caution is observed not to use  $\text{HgCl}_2$  in contact with metal. Below each mounted standard disk on paper note quantity of dried material that the dirt or filth on disk represents.

NOTE: An excellent set of Standard disks has been prepared and photographed by the Connecticut State Department of Health Laboratory. Thru the courtesy of the Connecticut laboratory, photographic copies of these standard disks may be

secured thru the office of the American Public Health Association, 1790 Broadway, New York City.

The standards given, based on pint samples of milk to which weighed amounts of sediment have been added, cover entire range from "clean" to "very dirty" milk. Numerical ratings are given for convenience of those who wish to use these standard disks as basis of percentage or other numerical scores. No attempt should be made to grade as sediment any hair, piece of hay or straw, or any large particle of dirt. These should be reported separately.

✓ **PHOSPHATASE TESTS FOR PASTEURIZATION (17)**

*Long Test—Tentative*

**22.43**

**COLLECTION OF SAMPLE**

Proceed as directed under 22.1, except to be assured that no preservative is present and not more than 48 hours has elapsed between time of sampling and receipt at laboratory. If samples are refrigerated, observe precautions to prevent freezing.

**22.44**

**REAGENTS**

(a) *Buffer substrate*.—Dissolve 1.09 g of disodium phenyl phosphate and 11.54 g of Na veronal (Na diethyl barbiturate) in  $H_2O$  saturated with  $CHCl_3$  and dilute to 1 liter. Add 10 ml of  $CHCl_3$ /liter and store reagent in refrigerator.

(b) *Folin-Ciocalteu phenol reagent*.—Dissolve 100 g of Na tungstate ( $Na_2WO_4 \cdot 2H_2O$ ), (according to Folin) and 25 g of Na molybdate ( $Na_2MoO_4 \cdot 2H_2O$ ) in 700 ml of  $H_2O$  in 1500 ml flask connected by ground-glass joint to reflux condenser. Add 50 ml of 85% phosphoric acid and 100 ml of HCl and reflux gently for 10 hours. Cool and add 150 g of  $Li_2SO_4$ , 50 ml of  $H_2O$ , and 4–6 drops of liquid Br. Boil mixture without condenser 15 min. to remove excess Br. Cool, transfer to liter flask, dilute to volume with  $H_2O$ , and filter. (Finished reagent should have golden yellow color; reject it if it has a greenish tint.) Keep reagent in refrigerator, protected from dust. For use dilute 1 volume of this stock reagent with 2 volumes of  $H_2O$ .

(c) *Sodium carbonate soln*.—Prepare a 14% or 1.32 M soln of anhydrous  $Na_2CO_3$ .

(d) *Filter paper*.—Must be free from phenol and other interfering substances (Whatman No. 40 and Eaton and Dikeman "New Filt," Nos. 1 and 3, have been found satisfactory).

**22.45**

**PERMANENT PHENOL STANDARDS**

(a) *Color soln, grey*.—Dissolve in  $H_2O$ , 31.9 g of  $CoCl_2 \cdot 6H_2O$ , 67.5 g of  $CuSO_4 \cdot 5H_2O$  and 75 g of  $NiSO_4 \cdot 6H_2O$ . Add 32 ml of HCl and 45 ml of  $H_2SO_4$  and dilute to 500 ml.

(b) *Color soln, red*.—Dissolve 476 g of  $CoCl_2 \cdot 6H_2O$  in  $H_2O$  and filter. To filtrate add 100 ml of HCl and dilute to 1 liter.

(c) *Color soln, blue*.—Dissolve 300 g of  $CuSO_4 \cdot 5H_2O$  in  $H_2O$ , add 20 ml of  $H_2SO_4$ , and dilute to 1 liter. (Should crystals appear when soln is cooled below 20°, warm slightly before using to insure complete solubility.)

Prepare permanent color standards equivalent to phenol concentrations of from 0.01 to 0.15 mg per 0.5 ml of sample by combining the quantities of color solns, a, b, and c indicated in 22.46 and diluting to 10 ml with  $H_2O$  in each case; e.g., 0.3 ml of Soln a + 0.106 ml of Soln b + 0.96 ml of Soln c +  $H_2O$  to make a volume of 10 ml is equivalent to a phenol concentration of 0.01 mg in 0.5 ml of sample.

These color standards are suitable for use only in natural daylight. If, however, a turquoise blue, unglazed, opaque glass plate is used to deflect the light from a day-

light lamp thru tubes of standards and sample, accurate color comparisons can be made in absence of daylight. Since the standards are prepared for use only at 13 mm depth of color, tubes of different diameter cannot be used for accurate work.

**22.46***Preparation of permanent phenol standards*

PHENOL	COLOR SOLUTION		
	GREY (a)	RED (b)	BLUE (c)
<i>mg/0.5 ml</i>	<i>ml</i>	<i>ml</i>	<i>ml</i>
0.01	0.30	0.106	0.96
0.02	0.40	0.140	1.16
0.03	0.55	0.180	1.65
0.04	0.65	0.216	2.10
0.06	0.92	0.286	3.00
0.09	1.30	0.326	4.40
0.12	1.70	0.360	5.70
0.15	2.50	0.396	7.10

**22.47**

## DETERMINATION

Transfer 10 ml of the buffer substrate soln into test tube 20×160 mm and add 0.5 ml of milk to be tested. Add a few drops of  $\text{CHCl}_3$ , mix thoroly by rotating tube, and cover to protect contents from dust. (Do not use rubber or cork stoppers; paper toweling placed over open end of tube is satisfactory.) Warm to 37–39° in water bath and incubate at 34–37° for not less than 18 and not more than 24 hours. After incubation add 4.5 ml of the diluted Folin-Ciocalteu reagent. Mix, and allow to stand 3 min. Filter, and transfer 5 ml of filtrate to test tube of 13 mm diam. Add 1 ml of the  $\text{Na}_2\text{CO}_3$  soln and mix thoroly by rotating tube. Place tube in boiling water bath for 5 min. and filter. Cool, and estimate color of filtrate by comparison with the permanent color standards.

**22.48**

## CONTROL TEST

(To check deterioration of reagents and presence of interfering substances in sample)

To 10 ml of the buffer substrate soln, add 4.5 ml of the Folin-Ciocalteu reagent and 0.5 ml of the milk sample. (Do not incubate.) Mix thoroly, allow to stand 3 min., and filter. To 5 ml of filtrate add 1 ml of the  $\text{Na}_2\text{CO}_3$  soln, mix thoroly by rotating tube, heat in boiling water bath 5 min., and filter. Cool, and compare color of filtrate with the permanent color standards. If phenol value obtained is greater than 0.02 mg, subtract excess from phenol value of incubated sample to obtain phenol value indicative of pasteurization treatment.

Phenol value of 0.04 mg of phenol per 0.5 ml of sample generally indicates milk heated to 143°F. for 30 min. Value greater than this indicates progressively inadequate heat treatment. In reporting results, give mg of phenol per 0.5 ml of sample as well as an interpretation as to whether the milk is pasteurized or under-pasteurized.

*Rapid Test—Tentative***22.49**

## COLLECTION OF SAMPLE

Proceed as directed under 22.1, except that if it is necessary to add a preservative only borax in amount of 0.8 g per 100 ml of milk may be used. If samples sour, neutralize to pH 6.6 with  $\text{Na}_2\text{CO}_3$  before proceeding with analysis.

## 22.50

## REAGENTS

(a) *Borate buffer*.—Dissolve 28.427 g of  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$  in 900 ml of  $\text{H}_2\text{O}$ . Add 3.27 g of  $\text{NaOH}$  (81.75 ml normal  $\text{NaOH}$  soln) and dilute to 1 liter.

(b) *Buffer substrate*.—Dissolve 0.5 g of *crystalline* disodium phenyl phosphate in 5 ml of  $\text{H}_2\text{O}$  in small (10×100 mm) test tube. Add 0.5 ml of borate buffer. Shake well and add 1/25 ml of the phenol reagent (or add 2 drops from dropper delivering 50 drops per ml of the phenol reagent). Shake well. Allow 5 min. for color development. Extract indophenol by shaking with 2 ml of neutral *n*-butyl alcohol. Allow to stand until alcohol has separated completely. Remove supernatant alcohol layer with pipet or medicine dropper and discard. Dilute remainder with 100 ml of borate buffer and sufficient  $\text{H}_2\text{O}$  to make 1 liter. (This buffer substrate is phenol-free.) Store under refrigeration. Because of possible decomposition, prepare quantities of this reagent sufficient for immediate needs only. The pH of this soln is ca 9.6 (blue to thymolphthalein soln—0.04% in 50% alcohol). Avoid intimate contact of soln with rubber. (Darkening indicates decomposition.)

(c) *Gibbs phenol soln*.—Dissolve 40 mg of 2,6 dibromoquinone-chloroimide in 10 ml of methyl or ethyl alcohol. Keep reagent tightly stoppered and under refrigeration.

(d) *Lead acetate soln*.—Dissolve 50 g of  $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$  in 100 ml of  $\text{H}_2\text{O}$ .

(e) *Sodium pyrophosphate soln*.—Dissolve 10 g of  $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$  in 100 ml of  $\text{H}_2\text{O}$ .

## 22.51

## PERMANENT PHENOL STANDARDS (ACID SERIES)

(a) *Color soln, red*.—0.5 *N*  $\text{CoCl}_2$  in 1%  $\text{HCl}$  (59.59 g of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ /liter).

(b) *Color soln, blue*.—30%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 1%  $\text{HCl}$  (300 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ /liter).

(c) *Color soln, yellow*.—0.5 *N*[*M*/6]  $\text{FeCl}_3$  in 1%  $\text{HCl}$  (45.04 g of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ /liter).

Combine quantities indicated in 22.52 and dilute to 5 ml with  $\text{H}_2\text{O}$ .

These color standards and those in 22.54 are suitable for use in natural or artificial light provided an opal double-fluxed glass or sheet of Plastacele No. C-1605 HH, 0.015" thick, is used as light filter. Use for color standards test tubes similar to those in which each test is conducted.

## 22.52

## Preparation of permanent phenol standards—acid series

UNITS	COLOR SOLUTIONS		
	BLUE (b)	RED (a)	YELLOW (c)
	<i>ml</i>	<i>ml</i>	<i>ml</i>
1	0.2	0.35	0.5
2	0.5	0.6	0.55
3.5	0.5	0.5	0.5
5	1.0	0.75	0.5
7.5	1.5	0.75	0.5
10	2.0	1.0	0.25

## 22.53

## PERMANENT PHENOL STANDARDS (AMMONIACAL SERIES)

(a) *Color soln, red*.—Dissolve 1.8 g of roseo (aquopentammine) cobaltic chloride [ $\text{Co}(\text{NH}_3)_5 \cdot \text{H}_2\text{O}] \text{Cl}_3$  per liter of 2.8%  $\text{NH}_4\text{OH}$ .

(b) *Color soln, blue*.—6.24 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  per liter of 2.8%  $\text{NH}_4\text{OH}$ .

(c) *Color soln, yellow*.—Dissolve 0.84 g of  $(\text{NH}_4)_2\text{CrO}_4$  per liter of 2.8%  $\text{NH}_4\text{OH}$ .

Combine quantities indicated in 22.54 and dilute to 5 ml with  $\text{H}_2\text{O}$ :

**22.54**      *Preparation of permanent phenol standards—ammoniacal series*

UNITS	COLOR SOLUTIONS		
	BLUE (b)	RED (a)	YELLOW (c)
	<i>ml</i>	<i>ml</i>	<i>ml</i>
1	0.25	0.5	0.4
2	0.5	0.75	0.5
3.5	0.8	1.0	0.7
5	1.0	0.6	0.55
7.5	1.25	0.75	0.75
10	1.5	0.25	0.5
15	2.5		0.5
20	2.75		0.5
25	3.0		0.5
50	3.75		0.5
100	4.0		0.5
500	4.5		0.5

**22.55**

## DETERMINATION

Transfer 1 ml of the milk to Pyrex test tube (15×125 mm) and add 10 ml of the buffer substrate. Mix thoroly. Warm to ca 40°, and place in incubator or water bath for 1 hour at 37–45° (41° preferred). After incubation, place tube in boiling H<sub>2</sub>O for 5 min. Cool on ice H<sub>2</sub>O. Add 0.1 ml of the Pb acetate soln. Shake immediately and well. (Proteins will coagulate and separate sharply. In some instances it may be necessary to add an additional 0.05 ml of Pb acetate.) Filter. To 5 ml of filtrate in test tube (6× $\frac{1}{4}$ " ), add 0.5 ml of the borate buffer. (Addition of a few drops of the Na<sub>2</sub>P<sub>2</sub>O<sub>7</sub> soln will clarify filtrate turbidity, if any.) Add 0.04 ml of the phenol reagent (2 drops from recommended dropper). Mix thoroly by rotating tube. After 15 min., estimate color by comparison with permanent color standards.

**22.56**

## CONTROL TEST

(To check deterioration of reagents and presence of interfering substances in sample)

To 5 ml of the buffer substrate, add 1/25 ml of the phenol reagent. (Development of blue color in 15 min. indicates substrate decomposition.) Or, incubate 10 ml of buffer substrate with 1 ml of a boiled milk sample. Proceed as directed under 22.55. (Intensity of color indicates extent of substrate decomposition.) To 9 ml of H<sub>2</sub>O add 1 ml of the borate buffer and 1 ml of milk sample. Mix well, place in boiling H<sub>2</sub>O for 5 min., and follow thru as directed under 22.55. Intensity of blue color indicates extent of indophenol color due to interfering substances in milk sample.

**22.57**

## INTERPRETATION

Phenol value of 2 units or less generally indicates milk heated to 143° F. for 30 min. Commercially pasteurized milk usually yields 0 to 1 unit because of added time exposure incurred by operating variations in preheating, filling, and emptying of tanks, etc. Value of 2 units or greater in commercially pasteurized milk indicates inadequate heat treatment.

**22.58**

VITAMIN D MILK.—See Chap. 36

## CREAM

**22.59**

## COLLECTION OF SAMPLE—OFFICIAL

Proceed as directed under 22.1. Analyze sample as soon as practicable, preferably not later than 3 days after taking.

## 22.60

## PREPARATION OF SAMPLE—OFFICIAL

Immediately before withdrawing portions for the determinations, mix sample by shaking, pouring, or stirring until it pours readily and a uniform emulsion has been secured. If sample is very thick, warm it to 30–35°, and mix. In case lumps of butter have separated, heat sample to 38° or, if necessary, to 50°, by placing in warm water bath. Thoroughly mix portions for analysis and weigh immediately. (In commercial testing for fat by the Babcock method, it may be advisable to warm all samples to 38–50° in water bath previous to mixing.) Avoid overheating sample, thereby causing cream to “oil off” (especially necessary in case of thin cream).

## 22.61

## LACTIC ACID—OFFICIAL.—See 22.8–22.13

## 22.62

## TOTAL SOLIDS—OFFICIAL

Proceed as directed under 22.14, using 2–3 g of the sample.

## 22.63

## ADDED WATER (18)—OFFICIAL

Proceed as directed under 22.33, but use the following formula to calculate percentage of added H<sub>2</sub>O:

$$W = \frac{\% \text{ Serum in Cream } (T - T')}{T}, \text{ in which } W = \text{percentage of added H}_2\text{O; } T = \text{freezing point of undiluted cream } (-0.550^\circ); T' = \text{observed freezing point of given sample; and } \% \text{ Serum} = 100\% - (\% \text{ fat} + \% \text{ protein}).$$

If protein is not determined it may be assumed to be 38% of solids-not-fat.

## 22.64

## ASH—OFFICIAL.—See 22.16

## 22.65

## TOTAL NITROGEN—OFFICIAL.—See 22.17

## LACTOSE

## 22.66

## Gravimetric Method—Official.—See 22.24

## FAT

## 22.67

## Roese-Gottlieb Method—Official

Using 5 g of sample and diluting with H<sub>2</sub>O to ca 10.5 ml, proceed as directed under 22.25, beginning “Add 1.25 ml of NH<sub>4</sub>OH”.

## Babcock Method—Official

## 22.68

## APPARATUS

(a) *Test bottles.*—Standard Babcock test bottles for cream shall be as follows:

(1) *50%, 9-g, short-necked, 6" cream-test bottle.*—Total height 150–165 mm (5.9–6.5"). Bottom of the bottle shall be flat, and axis of neck shall be vertical when bottle stands on level surface. Charge of cream for bottle shall be 9 g.

*Bulb.*—Capacity of bulb to junction with neck shall be not less than 45 ml. Shape of bulb shall be either cylindrical or conical. If cylindrical, outside diameter shall be 34–36 mm; if conical, outside diameter of base shall be 31–33 mm, and maximum diameter, 35–37 mm.

*Neck.*—Neck shall be cylindrical and of uniform diameter from at least 5 mm below lowest graduation mark to at least 5 mm above highest. Top of neck shall be flared to diameter of not less than 15 mm. Graduated portion of neck shall have length of not less than 63.5 mm. Total per cent graduation shall be 50. Gradua-



tions shall represent 5, 1, and  $\frac{1}{2}$ %, respectively, from 0.0 to 50%. The 5% graduations shall extend at least half-way around neck to right;  $\frac{1}{2}$ % graduations shall be not less than 3 mm in length; and 1% graduations shall be intermediate in length between 5% and  $\frac{1}{2}$ % graduations and shall project 2 mm to left of  $\frac{1}{2}$ % graduations. Each 5% graduation shall be numbered (thus: 0, 5, 10, . . . 45, 50), number being placed to left of scale. Capacity of neck for each whole per cent on scale shall be 0.1 ml. Maximum error in total graduation or any part thereof shall not exceed volume of smallest unit of graduation.

(2) *50%, 9-g, long-necked, 9" cream-test bottle.*—Same specifications shall apply to this bottle as to 50%, 9 g, 6" cream-test bottle, except that total height of this bottle shall be 210–229 mm (8.25–9.0") and graduated portion of neck shall have a length of not less than 120 mm.

(3) *50%, 18-g, long necked, 9" cream-test bottle.*—Same specifications shall apply to this bottle as to 50%, 9-g, 9" cream-test bottle, except that charge of cream for this bottle shall be 18 g.

Each bottle shall bear on top of neck above graduations, in plain legible characters, a mark denoting weight of charge to be used, *viz.*, "9 g" or "18 g," as case may be.

Each bottle shall be so constructed as to withstand stress to which it will be subjected in centrifuge.

(4) *Testing.*—Proceed as directed under 22.26(a).

(b) *Water bath for cream samples.*—Provided with thermometer and device for maintaining temp. of 38–50°.

(c) *Cream weighing scales.*—With sensibility reciprocal of 30 mg, *i.e.*, addition of 30 mg to either pan of scale, when loaded to capacity, shall cause deflection of at least 1 subdivision of graduation. Scales shall be set level upon table support and be protected from drafts.

(d) *Weights.*—9 g and 18 g, respectively, and plainly marked "9 g" or "18 g," as case may be. They shall be made of material capable of resisting corrosion or other injury, shall preferably be of low squat shape, with rounded edges, and shall be verified at frequent intervals by comparison with standardized weights.

(e) *Acid measure.*—See 22.26(c).

(f) *Centrifuge or "tester."*—See 22.26(d).

(g) *Dividers or calipers.*—See 22.26(e).

(h) *Water bath for test bottles.*—See 22.26(f).

## 22.69

### DETERMINATION

Weigh 9 g of prepared sample, 22.60, directly into 9-g cream-test bottle, or 18 g into 18-g bottle, and proceed by one of following methods:

*Method 1.*—After cream has been weighed into test bottle, add 8–12 ml of  $H_2SO_4$  (sp. gr. 1.82–1.83 at 20°) in case of 9 g bottle, or 14–17 ml in case of 18 g bottle, or add acid until mixture of cream and acid, after shaking, has assumed chocolate-brown color. Shake until all lumps have completely disappeared and add 5–10 ml of soft  $H_2O$  at 60° or above. Transfer bottle to centrifuge, counterbalance it, and after proper speed has been attained whirl 5 min. Add soft hot  $H_2O$  until liquid column approaches top graduation of scale; then whirl 1 min. longer at temp. of 55–60°. Adjust temp. as directed under 22.27, and with aid of dividers or calipers measure fat column, in terms of percentage by weight, from its lower surface to bottom of upper meniscus.

*Method 2.*—*For 9-g bottle only.*—After cream has been weighed into test bottle,

add 9 ml of soft  $H_2O$  and thoroly mix; add 17.5 ml of the  $H_2SO_4$  and shake until all lumps have completely disappeared. Transfer bottle to centrifuge, counterbalance it, and after the proper speed has been attained whirl 5 min. Fill bottle to neck with hot  $H_2O$  and whirl 2 min. Add hot  $H_2O$  until liquid column approaches top graduation of scale; and whirl 1 min. longer at temp. of 55–60°. Adjust temp. and measure fat column as directed under *Method 1*.

Whichever method is followed, fat column, at time of reading, should be translucent, of golden yellow to amber color, and free from visible suspended particles. All tests in which fat column is milky or shows presence of curd or of charred matter, or in which reading is indistinct or uncertain, should be rejected.

If glymol or pure white mineral oil (sp. gr. not to exceed 0.85 at 20°) is used, introduce a few drops only into bottle just before reading is made (it must not be dropped in, but must be allowed to flow down side of neck). For purpose of measurement, surface separating the glymol and fat is regarded as representing upper limit of column. Oil-soluble artificial color may be added to the white mineral oil.

**22.70** GELATIN—OFFICIAL.—See 22.34. Observe note

**22.71** PRESERVATIVES—OFFICIAL.—See 22.35 and Chap. 32

**22.72** COLORING MATTERS—OFFICIAL.—See 22.39 and Chap. 21

### EVAPORATED MILK (UNSWEETENED)

**22.73** PREPARATION OF SAMPLE—OFFICIAL

(a) Place unopened can in water bath at temp. of ca 60°. Remove, and shake can vigorously every 15 min. At end of 2 hours remove can and allow to cool to room temp. Remove entire lid and thoroly mix by stirring contents in can with spoon or spatula. (If separation of fat which has not become emulsified is noticed, sample preparation has been inadequate.)

(b) Dilute 40 g of prepared mixture (a) with 60 g of  $H_2O$  and mix thoroly.

**22.74** LACTIC ACID—OFFICIAL.—See 22.8–22.13

**22.75** TOTAL SOLIDS—OFFICIAL

Proceed as directed under 22.14, using 4–5 g of diluted sample, 22.73(b). Correct result for dilution.

**22.76** ASH—OFFICIAL

Ignite residue from total solids determination, 22.75, at temp. not above 550° until ash is free from C. Correct result for dilution.

**22.77** FAT—OFFICIAL

Weigh 4–5 g of undiluted sample, 22.73(a), into Mojonnier fat-extraction flask or Röhrig tube; dilute with  $H_2O$  to ca 10.5 ml; and proceed as directed under 22.25, beginning "Add 1.25 ml of  $NH_4OH$ ."

**22.78** TOTAL NITROGEN—OFFICIAL

Weigh 5 g of undiluted sample, 22.73(a), transfer to Kjeldahl flask, and proceed as directed under 2.24, 2.25 or 2.26. Per cent  $N \times 6.38$  = per cent "proteins."

## 22.79

## CASEIN—OFFICIAL

Weigh 10 g of diluted sample, 22.73(b), into beaker, and proceed as directed under 22.18 or 22.20. Correct result for dilution.

## 22.80

## ALBUMIN—OFFICIAL

Proceed as directed under 22.21, using filtrate from 22.79. Correct result for dilution.

## 22.81

## LACTOSE—OFFICIAL

Proceed as directed under 22.23 or 22.24, using diluted sample, 22.73(b), and correct result for the dilution.

## 22.82

## GELATIN—OFFICIAL.—See 22.34

## 22.83

## PRESERVATIVES—OFFICIAL.—See 22.35 and Chap. 32

## 22.84

## COLORING MATTERS—OFFICIAL.—See 22.39 and Chap. 21

## SWEETENED CONDENSED MILK

## 22.85

## PREPARATION OF SAMPLE—OFFICIAL

(a) If can is cold, place it in H<sub>2</sub>O at 30–35° until warm. Open, scrape out all milk adhering to interior of can, and after transferring to dish sufficiently large to permit stirring thoroly, mix until whole mass is homogeneous.

(b) Weigh 100 g of thoroly mixed sample into 500 ml flask, dilute to mark with H<sub>2</sub>O, and mix thoroly. If sample will not emulsify uniformly, weigh out separate portion of (a) for each determination.

## 22.86

## LACTIC ACID—OFFICIAL.—See 22.8–22.13

## 22.87

## TOTAL SOLIDS—OFFICIAL

Use 10 ml of prepared soln, 22.85(b), and proceed as directed under 22.14. Correct result for dilution.

## 22.88

## ASH—OFFICIAL

Evaporate 10 ml of prepared soln, 22.85(b), to dryness on water bath and ignite residue as directed under 34.9 or 34.10. Correct result for dilution.

## 22.89

## PROTEIN—OFFICIAL

Determine N as directed under 2.24, 2.25, or 2.26, using 10 ml of prepared soln, 22.85(b), and correct result for dilution. Per cent N  $\times 6.38$  = per cent total "protein."

## 22.90

## LACTOSE—OFFICIAL

Dilute 100 ml of prepared soln, 22.85(b), in 250 ml volumetric flask to ca 200 ml; add 6 ml of CuSO<sub>4</sub> soln, 34.33(a), and alkali soln of concentration and in proportion as directed in 22.24. Make up to mark, and mix thoroly. Filter thru dry filter and determine lactose as directed under 34.39. Correct result for dilution.

## 22.91

## FAT—OFFICIAL

Weigh accurately 4–5 g of prepared sample, 22.85(a), into Mojonnier fat-extraction flask or Röhrig tube; dilute with H<sub>2</sub>O to ca 10.5 ml, and proceed as directed under 22.25, beginning "Add 1.25 ml of NH<sub>4</sub>OH."

## SUCROSE (19)—OFFICIAL

## 22.92

## REAGENT

**Mercuric nitrate soln.**—To 220 g of yellow HgO, add 300–400 ml of H<sub>2</sub>O and sufficient HNO<sub>3</sub> to form a clear soln (ca 140 ml), being careful to use least possible excess of acid. Dilute to 800–900 ml and add 10% NaOH slowly and with constant shaking until slight permanent precipitate is obtained. Dilute to 1 liter and filter. As soln tends to become acid with age owing to deposition of basic Hg salts, dilute alkali should be added occasionally until slight permanent precipitate is formed and soln filtered.

## 22.93

## DETERMINATION

Introduce 50 ml of prepared soln, 22.85(b), into 100 ml flask; add 25 ml of H<sub>2</sub>O, mix, add 5 ml of the Hg(NO<sub>3</sub>)<sub>2</sub> soln, and shake thoroly. Without delay and while shaking constantly, add sufficient 0.5 *N* NaOH to render mixture neutral to litmus paper, being careful to avoid alkaline reaction (usually 12–13 ml will be required). Dilute to 100 ml with H<sub>2</sub>O, mix thoroly, and filter thru dry paper. Polarize filtrate in 200 mm tube, then invert at room temp. as directed under 34.24(c), and polarize inverted soln. Correct both readings for volume occupied by protein, 22.89, and fat, 22.91, 1 g of protein occupying space of 0.8 ml and 1 g of fat, 1.075 ml. Calculate percentage of sucrose by following formula, using corrected direct and invert readings obtained above:

$$S = \frac{100(a-b)}{142.35 - \frac{t}{2}} \times \frac{26}{W}, \text{ in which } S = \text{percentage of sucrose in the sample; } a = \text{corrected direct polarization; } b = \text{corrected invert polarization; } t = \text{temp. of soln polarized; and } W = \text{weight of sample taken (10 g).}$$

## DRIED MILK AND MALTED MILK

## 22.94

## SAMPLING DRIED MILK (20)—TENTATIVE

Use care to minimize any moisture absorption from air during sampling and avoid sampling on rainy day, or when humidity is high.

On surface of milk at top of barrel locate a point on each end of a diameter and on a radius perpendicular to this diameter, 1–2" in from edge of barrel. Midway on each side of triangle between these points locate a point. At the 6 points so located, using tubular trier sufficiently long to extend full length of barrel, draw a core parallel to vertical axis of barrel. Transfer cores to clean, dry, air-tight container and seal immediately.

Before opening sample for analysis, make homogeneous either by shaking or by alternately rolling and inverting container. Also avoid excessive temp. and humidity when opening sample container.

## 22.95

## PREPARATION OF SAMPLE—TENTATIVE

Sift sample thru 20-mesh sieve onto large sheet of paper, rubbing material thru sieve and tapping vigorously if necessary. Grind residue in mortar, pass thru sieve, and mix into sifted material. Discard particles of wood and other material that cannot be ground. Sift sample 2 more times, mixing thoroly each time. To avoid absorption of moisture, operate as rapidly as possible, and preserve sample in air-tight container.

**22.96****MOISTURE (21)—TENTATIVE**

Weigh 1–1.5 g of sample into round, flat-bottomed metal dish (not less than 5 cm in diam. and provided with close-fitting slip-in cover). Loosen cover and place dish on metal shelf (dish resting directly on shelf) in vacuum oven kept at temp. of boiling  $H_2O$ . Dry to constant weight (ca 5 hours) under pressure not to exceed 100 mm (4") of Hg. During drying admit into oven slow current of air (ca 2 bubbles/second), dried by passing thru  $H_2SO_4$ . Discontinue action of vacuum pump and carefully admit dried air into oven. Press cover tightly into dish, remove from oven, cool, and weigh. Calculate per cent loss in weight as moisture.

**22.97****PROTEIN—TENTATIVE**

Weigh 1 g of sample into Kjeldahl digestion flask and determine N as directed under 2.26. Per cent  $N \times 6.38$  = percent of "protein."

**22.98 CASEIN IN MALTED MILK AND CHOCOLATE MALTED MILK(22)—TENTATIVE**

Place 10 g sample in centrifuge bottle of 250 ml (or larger) capacity and extract with two 100 ml portions of petroleum benzine by shaking until uniform, centrifuging, and decanting supernatant layer. To dry residue add exactly 200 ml of 3%  $Na_2C_2O_4$  soln. Shake occasionally over 4-hour period. Centrifuge for 15 min. at high speed (1800 r.p.m. if Size 1 Sb centrifuge is used). Pipet 50 ml (100 ml for chocolate malted milk product) of supernatant liquid into 250 ml beaker. Add 50 ml of paper pulp soln (1 filter paper) and 2 ml of acetic acid dropwise with constant stirring. Set beaker in warm  $H_2O$  (45–50°) and let stand 15 min. Cool to room temp. and filter with moderate suction thru 7 cm Büchner funnel, previously fitted with No. 589 white ribbon paper and overlaid with layer of paper pulp. Wash precipitate 2 or 3 times with cold  $H_2O$ . (Filtrate should be clear, or nearly so. If first portions of filtrate are not clear, repeat filtration and complete washing of precipitate.) Determine N in washed precipitate and filter paper as directed in 2.26, and multiply by 6.38 to obtain equivalent of casein. Correct result for blank on reagents and paper pulp.

**22.99****ASH—TENTATIVE**

Ignite 1 g of sample at dull red heat until free from C. If suitable dish was used for moisture determination, 22.96, ash may be determined on portion there used. Cool in desiccator and weigh.

**22.100****ALKALINITY OF ASH IN DRY SKIM MILK—OFFICIAL**

Ash 2 g of dry skim milk for 1 hour at 550°. Add a few ml of  $H_2O$  to ash, break up with flattened stirring rod, evaporate to dryness over steam bath, and again ash for 1 hour. Again add a few ml of  $H_2O$  to ash, break up, and transfer to beaker with 50–75 ml of  $H_2O$ . Add 50 ml of 0.1 N HCl, heat to boiling, and boil gently for 5 min. Cool, add 30 ml of 40%  $CaCl_2$  soln (neutralized with 0.1 N HCl and filtered) and ca 10 drops of phenolphthalein indicator, and titrate excess acid with 0.1 N NaOH. Acid used (ml)  $\times 50$  = alkalinity of ash.

**22.101****FAT IN MALTED MILK (23)—TENTATIVE**

Weigh quickly ca 1 g of well-mixed sample into small, lipped beaker. Add 1 ml of  $H_2O$  and rub to smooth paste. Add 10 ml more of  $H_2O$ , warm on steam bath, and transfer to Mojonnier fat-extraction flask or Röhrig tube with aid of 10 ml of

alcohol. Mix thoroly, cool, and proceed as directed under 22.25, beginning "Add 25 ml of ether," rinsing beaker with this ether.

#### FAT IN DRIED MILK (24)—OFFICIAL

##### 22.102

##### PREPARATION OF SOLUTION

Proceed as directed in one of following methods:

(a) Weigh quickly ca 1 g of well-mixed sample into small, lipped beaker. Add 1 ml of  $H_2O$  and rub to smooth paste. Add 9 ml more of  $H_2O$  and 1 ml of  $NH_4OH$  and warm on steam bath. Transfer to Mojonnier fat-extraction flask or Röhrig tube. Cool, and proceed as directed in 22.103, rinsing beaker successively with the alcohol and ethers used in first extraction.

(b) Weigh quickly ca 1 g of well-mixed sample and transfer to Mojonnier fat-extraction flask or Röhrig tube. Add 10 ml of  $H_2O$  and shake until homogeneous, warming if necessary. Add 1 ml of  $NH_4OH$  and heat in water bath at 60–70° for 15 min., shaking occasionally. Cool, and proceed as directed in 22.103.

##### 22.103

##### DETERMINATION

Add 10 ml of alcohol, and mix. Extract with ether and petroleum benzine as directed under 22.25. For the second extraction add 4 ml of alcohol, and again extract as directed under 22.25. With whole milk and cream powders make third extraction, using 15 ml of each solvent after adding, if necessary, sufficient  $H_2O$  to bring aqueous layer in tube to original volume.

##### 22.104

##### CITRIC ACID IN DRIED MILK—TENTATIVE

Weigh 6 g of well-mixed sample, mix well with 44 ml of  $H_2O$ , and proceed as directed under 22.5, beginning "Add ca 100 mg of tartaric acid."

##### 22.105

##### LACTIC ACID—OFFICIAL.—See 22.8–22.13.

##### 22.106 MICROSCOPIC IDENTIFICATION OF MALTED MILK AND ITS FLAVORED PRODUCTS (25)—TENTATIVE

Mount small quantity of material in drop of mineral oil on slide, apply cover-glass, and examine preparation at magnification of ca 200, using microscope lamp with daylight glass as source of light. Control light intensity by iris diaphragm, because too brilliantly lighted field hinders recognition of details. (See Figs. 33 and 34.)

A represents spray-dried whole milk. Large particles represent aggregates of globular milk masses having stippled surface (a). Fat appears as droplets (b).

B represents a spray-dried malt extract having appearance of aggregates of droplets enclosed in spherical masses.

C represents a product made by mixing spray-dried whole milk and the spray-dried malt extract, shown in A and B, in proportion necessary to give approximate composition of malted milk. The globular stippled milk masses (b) and the malt extract masses (a) are easily recognized; (c) fat globules.

D represents spray-dried skim milk that might be confused with the spray-dried malt extract (B) because structure of spherical masses is similar. Comparison of the 2 pictures, however, will show that droplets in malt extract masses are larger than those appearing in milk masses.

E represents a product purchased on market and represented to contain malt, skim milk, whole milk, cocoa, and sugar. Examination shows malt extract (b), dried

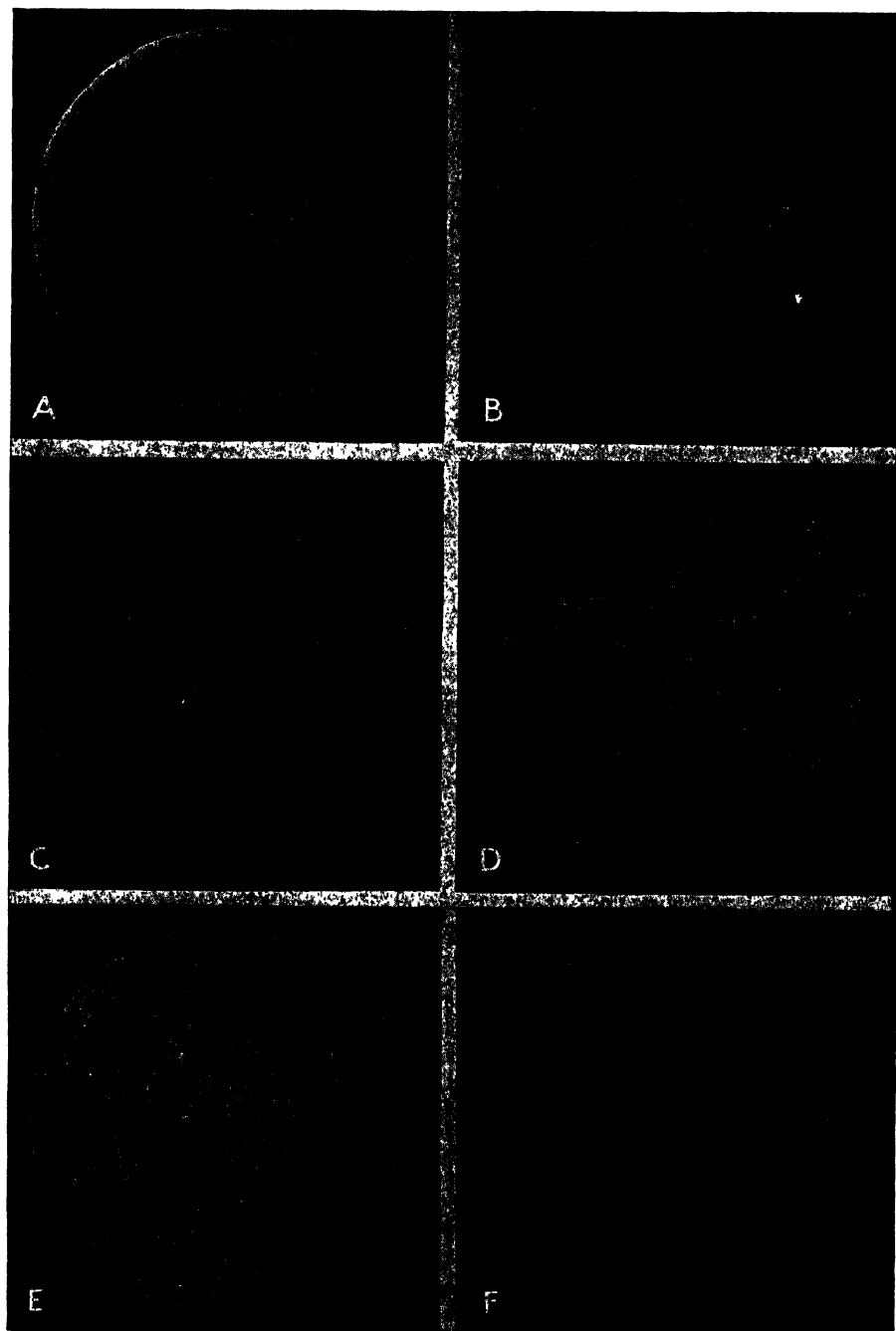


FIG. 33.—PHOTOMICROGRAPHS IDENTIFYING MALTED MILK AND ITS ALLIED PRODUCTS

skim milk (a), cocoa (c), and sugar (d) present. Whole milk is absent. The cocoa consists of brown amorphous particles, easily discernible under microscope. The highly refractive, irregular fragments of sugar cannot be mistaken.

F represents a drum-dried malt extract. It consists of clear, highly refractive fragments closely resembling broken glass.

A represents mechanical mixture of dried whole milk (A, Fig. 33), dried malt extract (B, Fig. 33), cocoa and sugar. The milk (a) and malt extract (b) masses and sugar particles (d) are readily recognizable. A mass of cocoa appears near center of picture (c).

B is characteristic of genuine malted milks on the market. This picture cannot be mistaken for any product of similar composition. The malt extract solids and the milk solids are incorporated into homogeneous irregular fragments having a stippled surface.

C represents a "sweet-chocolate-flavor malted milk" purchased on market, prepared by simultaneously evaporating in vacuo milk, malt infusion, cocoa and sugar. It is easy to recognize characteristic malted milk masses (a), shown in the picture immediately preceding. They are slightly thicker and appear darker in picture because the cocoa is intimately associated with them.

D represents a mechanical mixture of spray-dried skim milk (D, Fig. 33), spray-dried malt extract (B, Fig. 33), cocoa and sugar. No trouble is experienced in identifying these materials.

E represents a mechanical mixture of malted milk (B), cocoa, and sugar. Examination shows that malted milk (a) is present.

F represents a product found on market under label "malted milk." It shows none of characteristics found in genuine malted milk, as is readily seen by comparison with B. Individual milk masses (a), fat globules (c), and malt extract masses (b) closely resembling the spray-dried products (A and B, respectively, Fig. 33) predominate. Some particles show stippled surface of genuine malted milk, but they are spherical instead of angular. A comparison of this picture with picture showing mechanical mixture of spray-dried whole milk and spray-dried malt extract (C, Fig. 33) shows striking similarity.

## BUTTER

(Methods are also applicable to renovated or process butter and margarine)

### 22.107

#### SAMPLING (26)—TENTATIVE

(a) *Tub or cube butter.*—Insert regular trough butter trier practically its full length from point near top edge (or corner in case of cube) thru center to point at bottom diagonally opposite point of entry. Give trier one complete turn and withdraw full core. Hold point of trier over mouth of sample container and immediately transfer core of butter in ca 3" sections, working it from trier by aid of spatula fitted to groove. Leave plug ca 1" long to place in hole from which core was removed. Add two other trierfuls taken similarly at points equidistant from first (two other corners in case of cube) to the jar to constitute subdivision from tub or cube sampled.

#### DESCRIPTION OF THE PHOTOMICROGRAPHS

A.—SPRAY-DRIED WHOLE MILK: (a) MILK MASSES, (b) FAT GLOBULES; B.—SPRAY-DRIED MALT EXTRACT; C.—MECHANICAL MIXTURE: (a) SPRAY-DRIED MALT EXTRACT, (b) MILK MASSES, (c) FAT GLOBULES; D.—SPRAY-DRIED SKIM MILK; E.—MECHANICAL MIXTURE: (a) SPRAY-DRIED SKIM MILK, (b) SPRAY-DRIED MALT EXTRACT, (c) COCOA, (d) SUGAR; F.—DRUM-DRIED MALT EXTRACT.



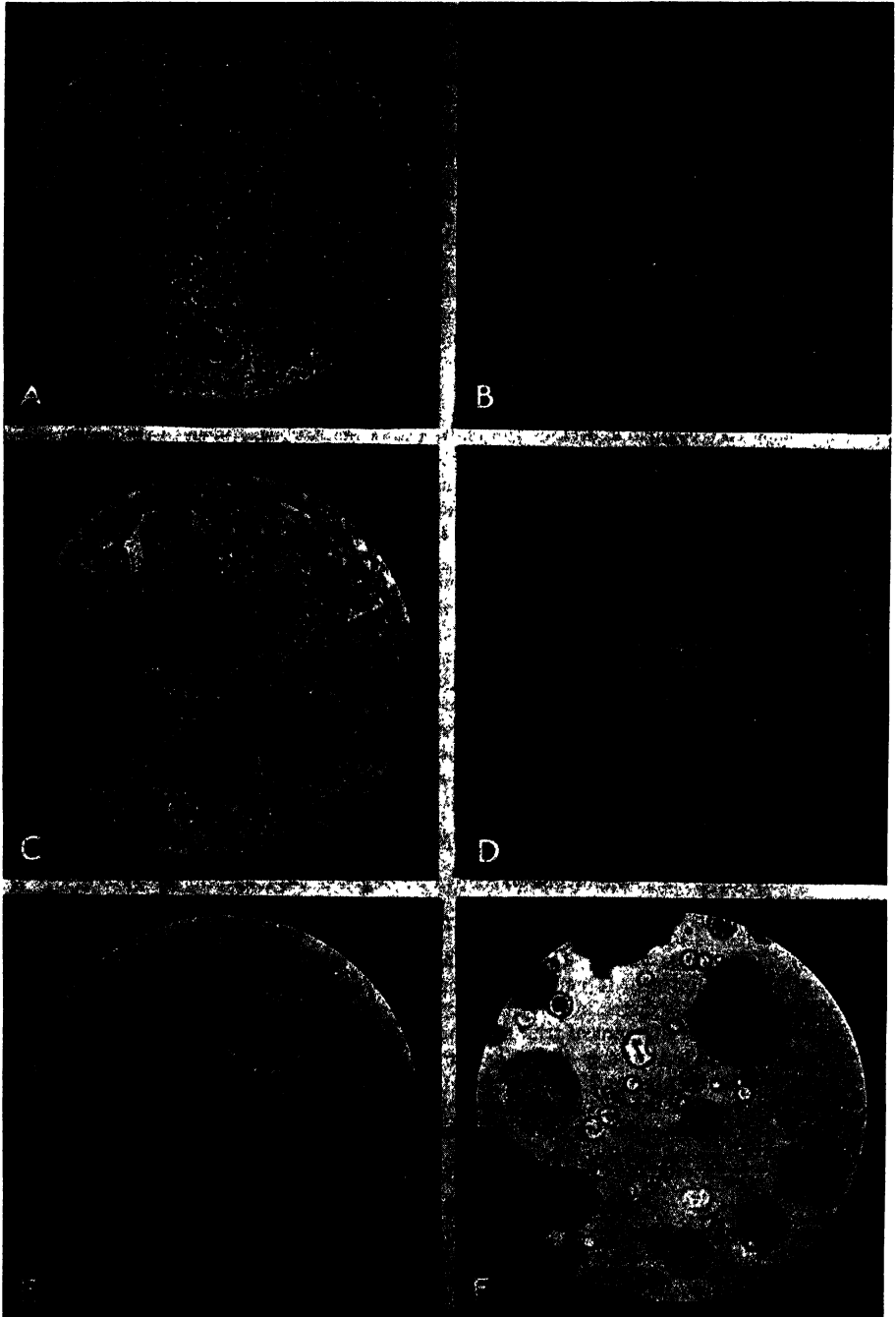


FIG. 34.—PHOTOMICROGRAPHS IDENTIFYING MALTED MILK AND ITS ALLIED PRODUCTS

Do not include moisture adhering to outside of trier. Clean and dry trier before each drawing. Use unwarmed trier for butter stored above freezing point. For harder butter use trier warmed to temp. that may be just borne by hand. Soften butter frozen so hard as to resist trier by storage in tempering room for 24 hours.

Sample lots as follows:

(1) *Tubs (or cubes) marked with churn numbers.*—Sample one tub of each churn of 1–9 tubs, two of each churn of 10–14 tubs, and three of each churn of over 14 tubs. In no case sample *less than* two tubs in a lot.

(2) *Tubs not marked with churn numbers.*—Sample number of tubs equivalent to square root of number in lot, with minimum of 3 and maximum of 25. If square root is not whole number, sample one extra tub.

(b) *Print butter.*—Withdraw one print from each of a number of cases equivalent to square root of number of cases in lot, with minimum of 5 and maximum of 25. When square root is not a whole number, sample one extra case. Select cases to include each churn or batch mark when so marked. With less than 5 cases sample all, taking 5 prints as minimum. Remove wrapper and transfer each print to separate sample container. (With prints of 1 pound or over, print may be quartered and two opposite quarters selected as sample.) With 8 oz. and 4 oz. prints, take whole print as sample.

These directions provide minimum sampling, to be increased if object of examination demands.

(c) *Sample containers.*—Use glass jar, preferably with glass top, of such type as will prevent loss of moisture by evaporation or entrance of H<sub>2</sub>O into jar. Tops containing a liner of any material should not be used.

#### PREPARATION OF SAMPLE (27)

##### 22.108

##### *Shaking Method—Official*

Soften entire sample in closed vessel by heating in water bath kept at 39°, and shaking occasionally until temp. of entire mass is 35°; or by heating in constant temp. oven at 35° until temp. of entire mass has reached 35°. Shake vigorously until homogeneous semi-solid mass is obtained. Weigh the portion for analysis at once. If sample is kept for any length of time, again soften and prepare as directed above before withdrawing portions for analysis.

##### *Mechanical Stirrer Method—Tentative*

##### 22.109

##### EQUIPMENT

(a) *Stirrer.*—Electric food mixer of double beater type with variable speed control and geared down to give maximum speed of 1000 r.p.m.; beater chucks should be on 1½" centers, and motor housing be fitted with clamp for use on ring stand.

(b) *Paddles.*—Brass, of dimensions given in Fig. 35. Ends of shafts should be

#### DESCRIPTION OF THE PHOTOMICROGRAPHS

A.—MECHANICAL MIXTURE: (a) MILK MASSES, (b) SPRAY-DRIED MALT EXTRACT, (c) COCOA, (d) SUGAR; B.—MALTED MILK; C.—SWEET-CHOCOLATE-FLAVOR MALTED MILK: (a) MALTED MILK MASSES, (b) SUGAR; D.—MECHANICAL MIXTURE: (a) SPRAY-DRIED SKIM MILK, (b) SPRAY-DRIED MALT EXTRACT, (c) COCOA, (d) SUGAR; E.—MECHANICAL MIXTURE: (a) MALTED MILK MASSES, (b) COCOA, (c) SUGAR; F.—PRODUCT PREPARED FROM MALT INFUSION AND MILK BY SPRAY DRYING: (a) MILK MASSES, (b) MALT EXTRACT MASSES, (c) FAT GLOBULES.

fitted to beater chucks so as to be easily removable; blades are made of 22 gage spring brass, soldered to slot in shaft.

(c) *Cover*.—Preferably made of light metal,  $4\frac{1}{2}$ " in diam., holes for shafts should be slightly oversize to permit cover to move easily up or down while paddles are turning.

## 22.110

## PREPARATION

Warm entire sample in sample jar, 22.107(c), until sufficiently softened to be readily stirred (25–30°). Insert paddles, with cover in place, and mix at high speed for 2–3 min. Raise, lower, and rotate jar until all portions of sample are incorporated in mixture. Remove paddles and replace jar top. Remix sample if weighing portion for analysis is delayed over 3 hours or after temp. goes above 30° or below 23°.

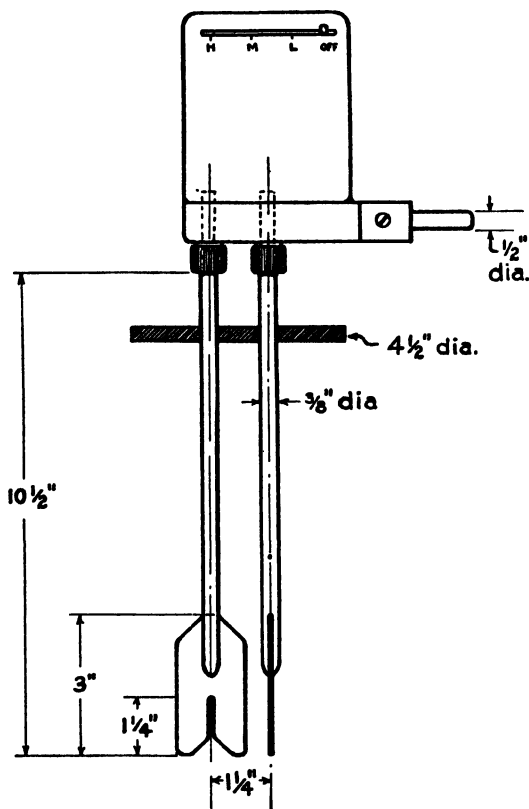


FIG. 35.—MECHANICAL STIRRER FOR PREPARATION OF BUTTER SAMPLES

ml of solvent. (Last 25 ml of the 100 ml of solvent should pass thru crucible without aid of suction.) Dry crucible and contents at temp. of boiling  $H_2O$  until weight is constant. Repeat washing with 25 ml of solvent and dry to constant weight. Repeat operation until there is no loss in weight due to washing.

## 22.113

*Direct Method—Official*

From the dry butter, obtained in determination of moisture either with or with-

22.111 **MOISTURE—**  
**OFFICIAL**

Weigh 1.5–2.5 g of prepared sample, 22.108, or 22.110, into flat-bottomed dish not less than 5 cm in diameter and dry to constant weight in oven kept at temp. of boiling  $H_2O$ . Clean, dry sand or asbestos may be used if fat is not to be determined in residue by 22.112.

**FAT (87)**22.112 *Indirect Method—*  
*Official*

Take up the dry butter, obtained in moisture determination in which no absorbent was used, 22.111, by macerating with 15 ml of absolute ether or petroleum benzene; transfer to weighed Gooch crucible with aid of wash bottle filled with the solvent; and wash free from fat with 100

out use of an absorbent, extract the fat with anhydrous, alcohol-free ether, or with petroleum benzine (b.p. below 65°), receiving soln in weighed flask. Evaporate solvent and dry extract to constant weight at temp. of boiling H<sub>2</sub>O.

**22.114 CASEIN, ASH, AND SALT—OFFICIAL**

Cover crucible containing residue from fat determination by indirect method, **22.112**, and heat, gently at first. Raise temp. gradually to not over 500°, remove cover, and continue heating until contents of crucible are white. Loss in weight represents casein, and residue in the crucible, mineral matter. Dissolve this mineral matter in H<sub>2</sub>O slightly acidified with HNO<sub>3</sub> and determine Cl, either gravimetrically as directed under **12.42**, or volumetrically as directed under **12.44**, and calculate the NaCl.

**22.115 SALT—OFFICIAL**

Weigh in counterpoised beaker 5–10 g of sample; add ca 20 ml of hot H<sub>2</sub>O; and after butter has melted transfer to separator. Insert stopper and shake for a few min. Let stand until all fat has collected on top of the H<sub>2</sub>O; then draw off H<sub>2</sub>O into flask, being careful to let none of fat globules pass. Again add hot H<sub>2</sub>O, rinsing beaker, and repeat extraction 10–15 times, using 10–20 ml of H<sub>2</sub>O each time. Washings will contain all but mere trace of NaCl originally present in butter. Determine quantity in whole or in aliquot of liquid by titration with standard AgNO<sub>3</sub>, using K<sub>2</sub>CrO<sub>4</sub> indicator.

**22.116 EXAMINATION OF FAT—OFFICIAL**

Melt butter and keep in dry place at ca 60° for 2–3 hours, or until H<sub>2</sub>O and curd have entirely separated. Filter clear, supernatant fat thru dry filter paper in hot water funnel or in oven at ca 60°. If filtered liquid fat is not perfectly clear, refilter. Determine physical and chemical constants as directed under Chap 31.

**22.117 COLORING MATTERS—OFFICIAL**

Pour ca 2 g of filtered fat, dissolved in ether, into each of 2 test tubes. Into one of tubes pour 1–2 ml of HCl (1+1) and into other about same volume of 10% NaOH soln. Shake tubes well and allow to stand. In presence of some azo dyes the test tube to which the acid has been added will show a pink to wine-red coloration, while the alkaline soln in the other tube will show no color. If, on the other hand, annatto or other vegetable color is present, the alkaline soln will be colored yellow, while no color will be apparent in the acid soln.

*General test.*—Proceed as directed under Chap. 21, particularly **21.3** and **21.16(b)**, for detection of oil-soluble coal tar dyes and annatto.

**22.118 LACTIC ACID—OFFICIAL.—See 22.8–22.13**

**22.119 PRESERVATIVES—OFFICIAL.—See 22.35 and Chap. 32**

**22.120 MICROSCOPIC EXAMINATION—OFFICIAL**

(a) Place on slide small portion of fresh unmelted sample taken from inside of the mass, add a drop of pure olive oil, apply cover-glass with gentle pressure, and examine with magnification of 120–150 diameters for crystals of lard, etc. Examine another portion of sample with polarized light and selenite plate without use of oil. Pure fresh butter will show neither crystals nor parti-colored field with selenite. Renovated butter or other fats melted and cooled and mixed with butter will usually present crystals and variegated colors with the selenite plate.

(b) For further microscopic study dissolve in test tube 3–4 ml of the fat in 15 ml

of ether. Close tube with loose plug of cotton wool and allow to stand 12–24 hours at 20–25°. When crystals form at bottom of tube, remove with pipet, glass rod, or tube; place on slide, cover, and examine under microscope. Crystals formed by later deposits may be examined in similar way. Compare with crystals obtained in same manner from samples of known purity.

#### RENOVATED BUTTER (88) AND OLEOMARGARINE

##### 22.121

##### *I. Foam Test—Tentative*

Heat 2–3 g of sample in either spoon or dish over small flame. True butter will foam copiously, whereas process butter will bump and sputter like hot grease, with little or no foaming. Oleomargarine behaves like process butter, but chemical tests will determine whether sample is oleomargarine or butter.

##### 22.122

##### *II. Melted Fat Test—Tentative*

Melt 50–100 g of butter or renovated butter at 50°. Curd from butter will settle, leaving clear supernatant fat; in case of renovated butter, supernatant fat remains more or less turbid.

#### CHEESE

##### 22.123

##### SELECTION AND PREPARATION OF SAMPLE—OFFICIAL

When cheese can be cut, take a narrow, wedge-shaped segment reaching from outer edge to center. Cut this segment into strips and pass 3 times thru sausage machine. When cheese cannot be cut, take sample with cheese trier. If only one plug can be obtained, take it perpendicularly to surface of cheese at point  $\frac{1}{3}$  distance from edge to center and extending either entirely or half way thru. When possible draw 3 plugs, 1 from center, 1 from point near outer edge, and 1 from point half-way between other two. For inspection purposes reject rind, but for investigations requiring absolute quantity of fat in cheese include rind in sample. Grind plugs in sausage machine (preferable method), or cut very finely and mix thoroly.

##### 22.124

##### MOISTURE (89)—OFFICIAL

Weigh 2–3 g of prepared sample, 22.123, into round flat-bottomed metal dish, not less than 5 cm in diameter and provided with close-fitting slip-in cover. In case of soft cheese and process cheese of high moisture content, weigh 1–2 g and partially dry on steam bath. Place loosely covered dish on metal shelf (dish resting directly on shelf) in vacuum oven, kept at temp. of boiling  $H_2O$ . Dry to constant weight (ca 4 hours) under pressure not to exceed 100 mm (4") of Hg. During drying admit into oven slow current of air (ca 2 bubbles per second) dried by passing thru  $H_2SO_4$ . Discontinue action of vacuum pump and carefully re-admit air into oven. Press cover tightly into dish, remove dish from oven, cool, and weigh. Express loss in weight as moisture.

##### 22.125

##### ASH (90)—OFFICIAL

Weigh into a Pt dish 3–5 g of prepared sample, 22.123, place on steam bath, and dry ca 1 hour. (If cheese is rich in fat, small amount of absorbent cotton may be placed in dish.) Ignite cautiously to avoid spattering and remove burner while fat is burning. When flame has died out, complete ignition in muffle at temp. not exceeding 550°.

## 22.126

## TOTAL CHLORIDES (SI)—OFFICIAL

Weigh ca 3 g of prepared sample, 22.123, into a 300 ml Erlenmeyer flask and add 25 ml of 0.1 *N* AgNO<sub>3</sub>, which is more than enough to combine with all the Cl. Add 10 ml of halogen-free HNO<sub>3</sub> and 50 ml of H<sub>2</sub>O and boil. As soln boils add ca 15 ml of 5% KMnO<sub>4</sub> soln in 5 ml portions. (Soln becomes yellowish and clear.) Cool; filter soln into 200 ml graduated flask, washing filter paper thoroly with H<sub>2</sub>O at ca 20°; and make to volume. Titrate excess AgNO<sub>3</sub> in 100 ml of clear soln with 0.1 *N* KSCN, using 2 ml of saturated soln of Fe alum as indicator. Run blank on reagents used, following same procedurc, except to add sugar to destroy excess of KMnO<sub>4</sub>. Calculate Cl found to NaCl.

## 22.127

## NITROGEN—OFFICIAL

Determine N in weighed portion (ca 2 g) of prepared sample, 22.123, as directed under 2.24, 2.25, or 2.26. Per cent N  $\times 6.38$  = per cent "protein."

## 22.128

## ACIDITY—OFFICIAL

To 10 g of prepared sample, 22.123, add H<sub>2</sub>O at 40° until volume equals 105 ml, shake vigorously, and filter. Titrate 25 ml portions of filtrate, representing 2.5 g of sample, with standard NaOH, preferably 0.1 *N*, using phenolphthalein. Express result in terms of lactic acid. 1 ml of 0.1 *N* NaOH = 0.0090 g of lactic acid.

## 22.129

## COLORING MATTERS—TENTATIVE

Extract 25–50 g of prepared sample, 22.123, with ether, remove ether by evaporation, and proceed as directed under 21.3 or 21.15.

## 22.130

## FAT—OFFICIAL

In small, narrow (or tall form) beaker rub to smooth liquid 1 g of prepared sample, 22.123, with 9 ml of H<sub>2</sub>O and 1 ml of NH<sub>4</sub>OH. Digest mixture at low heat until casein is well softened; neutralize with HCl, using litmus as indicator; and add 10 ml more of HCl. Add ca 0.5 g of sand, previously digested with HCl, to prevent bumping, and boil gently for 5 min., keeping beaker covered with watch-glass. Cool soln, transfer to Mojonnier fat extraction flask or Röhrig tube, rinse beaker with 25 ml of ether, and transfer ether rinsings to flask or tube, shaking thoroly. Add 25 ml of petroleum benzine (b.p. below 65°), shake thoroly, and proceed as directed under 22.25, beginning "Centrifuge Mojonnier flask 20 min."

## 22.131

## EXAMINATION OF FAT—OFFICIAL

(a) *Alkaline extraction.*—In large, wide-necked flask, treat ca 300 g of the cheese, cut into fragments the size of a pea, with 700 ml of 5% KOH soln at 20°, shaking vigorously to dissolve the casein. In 5–10 min., casein will be dissolved, and fat will rise to surface in lumps. Collect lumps of fat into as large a mass as possible by shaking gently. Pour cold H<sub>2</sub>O into flask until fat is driven up into neck, and remove by suitable means. Wash fat thus obtained with just sufficient H<sub>2</sub>O to remove residue of alkali that it may contain. Fat is not perceptibly attacked by alkali in this treatment, is practically all separated in short time, and is then easily prepared for chemical analysis by filtering and drying as directed under 22.116. Examine fat as directed under Chap 31.

(b) *Acid extraction.*—Pass cheese thru grinding machine, transfer to large flask, and cover with warm H<sub>2</sub>O, using 1 ml for every g of cheese. Shake thoroly and add H<sub>2</sub>SO<sub>4</sub> slowly and in small quantities, shaking after each addition of acid. (Volume

of  $\text{H}_2\text{SO}_4$  should equal volume of  $\text{H}_2\text{O}$  used.) Remove fat, which separates after standing a few min., by means of a separator; wash free from sulfate, filter, and dry as directed under 22.116. Examine fat as directed under Chap 31.

#### TARTARIC ACID (39)

##### 22.132

##### *Qualitative Test—Tentative*

To 5 g of ground cheese, 22.123, add 40 ml of  $\text{H}_2\text{O}$  at temp. of ca  $50^\circ$  and shake until cheese is thoroly broken up. Add 3 ml of 1%  $\text{H}_2\text{SO}_4$  soln and shake vigorously. Add 2 ml of 20% phosphotungstic acid soln and again shake vigorously. Let stand 5 min. and filter. To 25 ml of filtrate add sufficient saturated  $\text{Ba}(\text{OH})_2$  soln to make alkaline and 25 ml of alcohol, shake vigorously, and allow to settle. Filter thru Büchner funnel, using light suction, and wash residue on filter several times with  $\text{H}_2\text{O}$ . Transfer portion of paste to small evaporating dish and dry on steam bath. Add a few ml of  $\text{H}_2\text{SO}_4$  and a few crystals of resorcin, and heat slowly. If tartaric acid is present, a rose-red color is produced that is slowly discharged on dilution with  $\text{H}_2\text{O}$ .

##### *Quantitative Method (33)—Official*

##### 22.133

##### REAGENTS

(a) *Potassium chloride wash soln.*—Dissolve 15 g of KCl in 100 ml of  $\text{H}_2\text{O}$  and add 20 ml of alcohol.

(b) *Tartaric acid soln.*—Dissolve 1.5 g of pure tartaric acid in previously boiled and cooled  $\text{H}_2\text{O}$  and dilute to 100 ml at  $20^\circ$ . Titrate with 0.1 N NaOH to determine quantity of tartaric acid in 10 ml of soln.

##### 22.134

##### DETERMINATION

Weigh 25 g of prepared sample, 22.123, into 500 ml salt-mouth bottle and add, 25 ml at a time, 100 ml of  $\text{H}_2\text{O}$  at  $50\text{--}60^\circ$ , shaking vigorously after each addition. If necessary, continue shaking until cheese is thoroly broken up. Add 25 ml of 2% Na oxalate soln and shake vigorously 1 min. Add 100 ml of 2% HCl, 25 ml at a time, shaking vigorously after each addition. Add 50 g of powdered KCl, and shake 5 min. To avoid churning, keep mixture warm (ca  $50^\circ$ ) during shaking. Transfer contents of bottle, with aid of  $\text{H}_2\text{O}$ , to 300 ml flask, cool to  $20^\circ$ , and make to mark with  $\text{H}_2\text{O}$ . Mix thoroly; let stand 10 min., with occasional shaking; and filter thru dry folded filter, discarding first few ml of filtrate. Disregard any opalescence and transfer 200 ml of filtrate to 250 ml flask. Neutralize with N NaOH, using phenolphthalein, and add 5.2 ml in excess. Make to mark with  $\text{H}_2\text{O}$ , mix thoroly, let stand a few minutes, and filter thru dry folded filter, discarding first few ml of filtrate. To 100 ml of filtrate in 250 ml beaker add, with constant stirring, 10 ml of the tartaric acid soln, 2 ml of acetic acid, and 23 ml of alcohol. Cool in ice bath, stir vigorously until the cream of tartar begins to crystallize, and let stand in refrigerator overnight. Prepare Caldwell crucible with pad of asbestos ca 10 mm thick. Decant most of liquid thru this filter, wash precipitate into crucible with the KCl wash soln, and wash beaker and precipitate 3 times, using total quantity of 20–30 ml of the wash soln. Place asbestos and precipitate in beaker in which precipitation was made and wash crucible with ca 50 ml of hot  $\text{H}_2\text{O}$ . Heat soln to boiling and titrate while hot with 0.1 N NaOH, using phenolphthalein. Calculate percentage of tartaric acid in cheese by means of formula:

$X = 14.26[0.015(B + 1.5) - A]$ , in which

$A$  = g of tartaric acid in 10 ml of the tartaric acid reagent; and

$B$  = ml of 0.1 *N* NaOH required for titration.

In the factor 14.26 the concentration caused by insoluble solids of cheese of average composition is also taken into consideration.

#### CITRIC ACID (34)

##### 22.135

##### *Qualitative Test—Tentative*

To 10 g of prepared sample, 22.123, add 20 ml of  $H_2O$  at ca  $50^\circ$  and shake vigorously until cheese is thoroly broken up. Add 20 ml of  $H_2SO_4$  (1+1) and 2 ml of 20% phosphotungstic acid soln, and shake vigorously. Let stand for 5 min. and filter. To 20 ml of filtrate add 10 ml of Br water and 5 ml of KBr soln (15 g in 40 ml of  $H_2O$ ) and proceed with oxidation as directed in 22.136. Add sufficient  $FeSO_4$  soln to dissolve precipitated  $MnO_2$ . If citric acid is present, heavy white precipitate that settles rapidly is formed.

##### 22.136

##### *Quantitative Method (35)—Official*

Weigh 25 g of prepared sample, 22.123, into 500 ml salt-mouth bottle, and add, 25 ml at a time, 100 ml of  $H_2O$  at  $50$ – $60^\circ$ , shaking vigorously after each addition. Continue shaking until cheese is thoroly broken up. Add 25 ml of 2% Na oxalate soln and shake vigorously 1 min. Add 100 ml of 1%  $H_2SO_4$ , 25 ml at a time, shaking vigorously after each addition. Add 3 ml of 20% phosphotungstic acid soln and shake; then add 25 g of powdered anhydrous  $Na_2SO_4$ , and shake 5 min. To avoid churning, keep mixture warm (ca  $50^\circ$ ) during shaking. Transfer contents of bottle with aid of warm  $H_2O$  to 300 ml volumetric flask, cool to  $20^\circ$ , and make to mark with  $H_2O$ . Mix thoroly, shake occasionally during 10 min., then filter thru dry folded filter, discarding first few ml of filtrate. Heat 200 ml of filtrate to boiling and while still hot add 20 ml of  $H_2SO_4$  (1+1) and 2 ml of the phosphotungstic acid soln. Mix and allow to stand 15 min. With aid of  $H_2O$  transfer mixture to 250 ml flask, cool to  $20^\circ$ , make to mark with  $H_2O$ , and filter thru dry folded filter. Transfer 100 ml of clear filtrate to 500 ml Erlenmeyer flask (ca 0.3 g of washed and dried asbestos may be added). Add 10 ml of freshly prepared saturated Br water and 5 ml of KBr soln (5 g KBr in 40 ml of  $H_2O$ ), mix thoroly, and heat to  $48$ – $50^\circ$ . Hold at this temp. for 5 min., add 25 ml of 5%  $KMnO_4$  soln, shake, and allow to stand ca 5 min. Cool flask and contents to ca  $8^\circ$ , add 40 ml of cold  $FeSO_4$  soln (20 g  $FeSO_4 \cdot 7H_2O$  in 100 ml of  $H_2O$  and 1 ml of  $H_2SO_4$ ), shake continuously 5 min., and let mixture stand overnight in refrigerator. Decant supernatant liquid thru Gooch crucible, measure volume of filtrate (a) and wash precipitate from Erlenmeyer into crucible with this filtrate. Wash precipitate with 3 successive 20 ml portions of ice-cold  $H_2SO_4$  (1+100), sucking dry after each addition, and finally wash with 3 successive 20 ml portions of ice-cold  $H_2O$ . Dry precipitate to constant weight over  $H_2SO_4$  in vacuum desiccator, protecting precipitate from strong light or, to save time, dry in current of air passed thru  $H_2SO_4$ . Weigh, and remove pentabromacetone by extracting first with 3 successive 20 ml portions of alcohol and then with 3 successive 20 ml portions of ether. Dry, and weigh crucible. To weight of pentabromacetone add 0.004 g for each 100 ml of filtrate (a) to compensate for solubility of pentabromacetone and multiply result by 6.06 to obtain percentage of anhydrous citric acid in cheese. (In this factor consideration is taken of concentration caused by insoluble solids in 25 g of cheese. It is assumed that solids of cheese are almost insoluble under conditions maintained and that average process cheese contains ca 60% solids. No allow-



ance is made for variation in salt or moisture content or for variation in specific volume of solids, as such variations do not appreciably affect results.)

#### LACTOSE IN PROCESS CHEESE (36)—TENTATIVE

##### 22.137

##### *Quantitative Method*

Weigh 25 g of prepared sample, 22.123, into 500 ml salt-mouth bottle, and add in 25 ml portions 100 ml of  $H_2O$  at 50–60°, shaking vigorously after each addition. Continue shaking until cheese is thoroly broken up. Add 25 ml of 2% Na oxalate and shake vigorously 1 min.; add 25 g of powdered  $Na_2SO_4$  and shake 2 min.; add 10 ml of  $H_2SO_4$  (1+1) and shake; then add 25 ml of 20% phosphotungstic acid soln and shake vigorously. Transfer contents of bottle to 500 ml flask, cool at once to 20°, and make to mark with  $H_2O$ . Mix thoroly, allow to stand 10 min., and filter thru dry folded filter. Transfer 150 ml of filtrate to each of two 250 ml flasks, add 10% NaOH soln to one flask until mixture is alkaline to litmus, then add 5 g of solid KCl and mix thoroly. Cool to 20° and make to mark with  $H_2O$ . Shake well, allow to stand 10 min., and filter thru dry folded filter. Determine lactose in 50 ml aliquot as directed in 34.39. Treat 50 ml of the 150 ml in the second flask as directed in 34.24(c), using 10 ml of HCl, etc. Add 10% NaOH soln until alkaline to litmus, and add 5 g of solid KCl. Mix thoroly, cool to 20°, and make to mark with  $H_2O$ . Let stand 10 min. Filter if necessary thru dry filter paper. Determine lactose in 50 ml aliquot as before. Agreement between amounts of  $Cu_2O$  reduced before and after inversion establishes absence of sucrose.

Since insoluble material of cheese and phosphotungstic acid precipitate occupy some space in flask as originally made up, it is necessary to correct for this volume. From average composition of cheese, volume of precipitate was calculated to be 14 ml. To obtain the true amount of lactose present, multiply all results by factor 0.97.

#### GUMS IN SOFT CURD CHEESE (37)—TENTATIVE

##### 22.138

##### REAGENTS

(a) *Benedict soln (qualitative)*.—Dissolve 17.3 g of Na citrate and 10 g of anhydrous  $Na_2CO_3$  in ca 80 ml of hot  $H_2O$ ; dissolve 1.73 g of  $CuSO_4 \cdot 5H_2O$  in 10 ml of  $H_2O$ . Filter the alkaline citrate soln, add the  $CuSO_4$  soln slowly, with constant stirring, and dilute with  $H_2O$  to 100 ml.

(b) *Sodium hydroxide soln*.—10%.

(c) *Trichloroacetic acid soln*.—50%. Use fresh soln made up just before use from non-hydrolyzed reagent.

(d) *Dilute trichloroacetic acid soln*.—10%. Note warning under (c).

(e) *Ammonium hydroxide*.—Reagent, 28–29%  $NH_3$ .

(f) *Acetic acid*.—Reagent, glacial, 99.5%.

(g) *Alcohol*.—70%.

##### 22.139

##### PREPARATION OF SAMPLE

Transfer 100 g of cheese to 500 ml casserole, add just sufficient  $H_2O$  to barely cover cheese and heat on steam bath for at least 30 min. If fat separates, remove casserole, add petroleum benzine, swirl, and decant off benzine. Repeat benzine treatment, after an interval of heating, until most of fat is removed. Warm defatted cheese on steam bath to remove residual petroleum benzine, add 90 ml of hot  $H_2O$ , and bring to boil while stirring continuously. Remove heat and add, with constant stirring, sufficient  $NH_4OH$  to bring pH to ca 9 (dark green with alkacid test paper). Allow to stand until all curd has dissolved (10–20 min.). Stir and macerate, if nec-

essary, to secure soln. Transfer dissolved material to 250 ml centrifuge bottle with 4–5 ml of  $H_2O$  and add acetic acid in small amounts, with shaking, until pH is ca 4.75 (nitrazine test paper). Use care in approaching pH point because isoelectric point for casein is ca pH 4.73. (If acid is added very slowly with constant shaking, marked separation of casein and liquid will be noted at this point.) Stopper bottle, shake thoroly, and allow to stand several min. (overnight causes no harm). Centrifuge at 1800 r.p.m. for 10 min. and decant liquid into 250 ml beaker. Do not wash precipitate.

**22.140****SEPARATION OF GUM**

Evaporate to ca 40 ml the separated gum soln obtained by centrifuging. Check volume carefully against 40 ml of  $H_2O$  in another 250 ml beaker. Disregard precipitate formed during concentration and add 10 ml of the trichloroacetic acid soln (note warning). Hold at 70° until precipitate coagulates (10–20 min.), avoiding prolonged heating, which will hydrolyze the gum. Cool, transfer to centrifuge bottle with 5 ml of the dilute trichloroacetic acid, and centrifuge. Decant supernatant liquid into another 250 ml centrifuge bottle and add alcohol with stirring until bottle is full. Allow mixture to stand 2–3 hours, if necessary, to coagulate gums. Centrifuge, and decant off liquid.

If supernatant liquid from trichloroacetic acid treatment is very cloudy, incomplete removal of protein may be indicated. In such a case add 40 ml of hot  $H_2O$  to precipitate in bottle, shake, and let stand at 70° until gum has been dissolved. Some protein may not dissolve. Then repeat gum separation beginning, "add 10 ml of trichloroacetic acid."

Add to bottle ca 50 ml of 70% alcohol, stopper, and shake to thoroly break up residue. Wash down stopper and sides of bottle with a little 70% alcohol, centrifuge, and again decant. Dissolve residue in 40 ml of hot  $H_2O$  and reprecipitate in bottle by filling with alcohol plus 2 drops of acetic acid. (This reprecipitation removes traces of sugar occluded by first precipitation.)

The very small alcohol precipitate obtained on cheese to which no gum has been added, caused by natural substances in the cheese which were not removed by precipitating agents used, should not be confused with the much more voluminous precipitates obtained when gum is present.

**22.141****DETECTION OF GUM**

Allow precipitate to coagulate, centrifuge, and decant. Add to residue 8–10 ml of alcohol, stopper bottle, and shake to break up residue. Transfer residue quantitatively with minimum quantity of alcohol to Hart casein tube and centrifuge at 1800 r.p.m. for 15 min. Read volume occupied by alcohol precipitate in casein tube. (Alcohol precipitate from a soft curd cheese with no added gum should occupy volume of only 0.1–0.2 ml. Precipitate from such a cheese with 0.05% added gum should occupy ca 10 times that volume.)

Add to casein tube 10 ml of hot  $H_2O$ , shake, and transfer to 50 ml beaker. Rinse out tube with 10 ml of hot  $H_2O$  and add to beaker. Warm to dissolve gum and evaporate to 10 ml. Add 2 ml of HCl. Boil 60 seconds. Transfer 1 ml of the hydrolyzed sugar soln to test tube; neutralize with the NaOH soln, using litmus paper as indicator, remove litmus paper; add 5 ml of the Benedict soln; and boil vigorously 2 min. Allow to cool spontaneously. Voluminous precipitate appearing on cooling, which may be yellow, orange, or red, caused by reducing sugars formed by hydrolysis of the gums, indicates presence of gums.

## GELATIN IN COTTAGE CHEESE

22.142

*Qualitative Test—Official*

Mix thoroly 5 g of sample with 10 ml of  $H_2O$  at 50–60° and add 5 ml of  $Hg(NO_3)_2$  soln, 22.34. Shake, allow to stand 5 min., and filter thru medium fast retentive paper. To filtrate add 5 ml more of the  $Hg(NO_3)_2$  soln and test as directed in 22.34, using filtrate so obtained. See also note under 22.34.

## ICE CREAM AND FROZEN DESSERTS

## WEIGHT PER UNIT VOLUME OF PACKAGED ICE CREAM—TENTATIVE

22.143

## REAGENTS

(a) *Kerosene*.—Of known density at 20/4°. Cool to 5–10° (icebox temp.) before use.

(b) *Dry ice*.

22.144

## APPARATUS

(a) *Overflow can*.—A No. 10, or 1 gallon can with overflow spout of  $\frac{1}{4}$ – $\frac{3}{16}$ " I.D. metal tubing soldered to opening in side of can ca 1" from bottom and bent upward and extending parallel to sides. The tube should be bent over at upper end to form a spout ca  $1\frac{1}{2}$ " below top of can. The upper edge of opening of spout should be above and the lower edge below highest point of interior surface of the top bend (A). An iron bar, slightly longer than diam. of can, equipped with a "bridge" of tinned metal may be used to submerge sample in the kerosene. The "bridge" should extend  $\frac{1}{4}$ " below level of A, Fig. 36.

(b) *Balance*.—Capacity 1 kg, sensitive to 1 g.

(c) *Cylinders or beakers*.—500 or 1000 ml graduated.

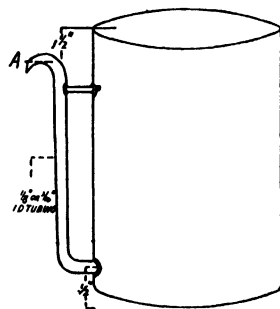


FIG. 36—OVERFLOW CAN

22.145

## DETERMINATION

Obtain packaged samples (pints preferred) from freezing compartment or cold room and immediately place in insulated container with dry ice, for transportation to laboratory. Surround package of ice cream with slabs or pieces of dry ice until frozen solid.

Place the overflow can on level table so that overflow will discharge into a sink. Fill can with the cooled kerosene until it overflows thru the spout. When overflow has ceased, place tared 500 ml graduated cylinder (or beaker) under spout.

Remove frozen brick from dry ice, quickly remove from carton, and weigh to accuracy of 1–2 g. Designate this weight as  $W$ . Slowly immerse brick into kerosene, finally completely submerging it by holding it under surface with small spatula, until overflow has ceased. Weigh displaced kerosene to accuracy of 1–2 g, and subtract tare weight of cylinder or beaker to ascertain net weight of kerosene displaced. Divide net weight of kerosene by its sp. gr. and designate resultant volume as  $V$ .

$$\text{Weight/unit volume as } \frac{\text{lbs.}}{\text{gals.}} = \frac{W \times 8.345}{V}$$

If ice creams are so packed that it is difficult to remove from carton, ascertain gross weight of carton and ice cream, then open ends or sides of carton sufficiently to avoid formation of entrapped air bubbles, and submerge entire carton and contents in the kerosene as directed. After overflow has ceased, and the displaced kerosene has been weighed, remove ice cream from carton, dry empty carton, and weigh. Transfer kerosene to 100 ml or 200 ml graduated cylinder, filling to half-way mark, and record volume. Roll up dried carton so that it will slip into the cylinder, avoiding entrapment of air. Push carton into cylinder until it is completely immersed in kerosene. Increase in volume is volume occupied by carton. Correct for weight and volume of carton in formula given above and calculate unit weight in lbs./gals.

A graduated cylinder may be used instead of beaker to catch overflow. The volume reading may be used as a check against the calculated volume of the kerosene. The volume as calculated from the weight is more nearly accurate.

**22.146****PREPARATION OF SAMPLE**

(a) *Plain ice cream (Official).*—Allow sample to soften at room temp. Because melted butter fat tends to separate and rise to surface, it is not advisable to soften the ice cream by heating on water bath or over flame. Mix thoroly by stirring with spoon or egg beater or by pouring back and forth between beakers.

(b) *Frozen desserts containing insoluble particles (Official, First Action).*—Use malted milk mixer capable of comminuting the ice cream to fine, uniform pulp. (Many satisfactory commercial mixers are on the market.)

Use sufficient sample (4–8 oz) to fill cup of mixer  $\frac{1}{4}$ – $\frac{1}{2}$  full. Melt at room temp. or in incubator set at  $37.5^{\circ}$ , in closed container (Mason jar is suitable), transfer entire sample to mixer cup, and mix until insoluble particles are finely divided (2–5 min. for fruit ice creams, and up to 7 min. for nut and certain candy ice cream). Transfer mixed sample to suitable container for convenience in weighing.

**22.147****TOTAL SOLIDS—OFFICIAL**

Into round flat-bottomed dish not less than 5 cm. in diam., weigh quickly 1–2 g of sample. (Sample may be weighed by means of short, bent, 2 ml measuring pipet.) Heat on steam bath 30 min., then in air oven at  $100^{\circ}$  for  $3\frac{1}{2}$  hours. Cool in desiccator and weigh quickly to avoid absorption of moisture.

**22.148****NITROGEN—OFFICIAL**

Proceed as directed in 2.26, using 4–5 g sample. Per cent N  $\times 6.38$  = per cent “protein.”

**FAT****22.149****Roese-Gottlieb Method—Official**

Weigh 4 g of thoroly mixed sample into small dry beaker, add 3 ml of  $H_2O$ , mix thoroly, and transfer to Röhrig tube or similar apparatus, washing out remaining portion with aid of an additional 3 ml of  $H_2O$ . Add 2 ml of  $NH_4OH$ , mix thoroly, and heat in water bath at  $60^{\circ}$ . Proceed as directed under 22.25, beginning “Add 10 ml of alcohol and mix well.”

**22.150****LACTIC ACID—OFFICIAL.—See 22.8–22.13****22.151****GELATIN—OFFICIAL**

Using 10 g sample, proceed as directed under 22.34.

## 22.152

## COLORING MATTERS—OFFICIAL

Curdle 150–200 g of melted sample by adding equal volume of  $H_2O$  and 10–20 ml of acetic acid. Heat mixture to 70–80°, stirring meanwhile, and allow to cool. Continue as directed under 22.39 and 22.117, and under Chap. 21, particularly 21.3 and 21.16(b) for detection of oil-soluble coal tar dyes and annatto.

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## 23. EGGS AND EGG PRODUCTS

### 23.1 COLLECTION AND PREPARATION OF SAMPLE (1)—TENTATIVE

No simple rules can be made for the collection of a sample representative of the average of any particular lot of egg material, as conditions may differ widely. Experienced judgment must be used in each instance. If large lots are under examination, it is best to draw a number of samples for separate analyses rather than to attempt to get one composite representative sample.

(a) *Liquid eggs*.—Secure representative container or containers. Mix contents of a container thoroly and draw ca 300 g. A long-handled dipper or ladle serves well. Keep sample in hermetically sealed jar in cool place. Report odor and appearance.

(b) *Frozen eggs*.—Secure representative container or containers. Examine contents as to odor and appearance. The condition of contents can be determined best by boring a hole to center of a container with auger and noting odor as the auger is withdrawn. If impossible to secure individual containers, samples may consist of the composite of the borings from the contents of each container. Take borings midway between center and circumference of top of can from at least 3 widely separated parts and extend them as near to bottom of can as possible. Collect ca 300 g of the sample. Keep sample in hermetically sealed jar in cool place and in frozen state if possible. Before analyzing warm sample in bath held below 50° and mix well.

(c) *Dried eggs*.—Secure representative container or containers. For small packages, take entire parcel or parcels for the sample. For boxes and barrels, remove top layer to depth of ca 6" with flour scoop or other convenient instrument. Draw small quantities of sample totaling 300–500 g from accessible parts of container and place in hermetically sealed jar. Report odor and appearance. Prepare sample for analysis by mixing 3 times thru domestic flour sifter to assure complete breaking up of lumps. Keep in hermetically sealed jar in cool place.

(d) *Flaked and drum-dried eggs*.—Collect sample as directed for powdered dried eggs. Report odor and appearance. Prepare albumin samples for analysis by grinding in mill to pass entirely thru 60-mesh sieve, and whole egg and yolk samples to pass entirely thru 20-mesh sieve or as fine as is practicable. Keep in hermetically sealed jar in cool place.

#### TOTAL SOLIDS

##### *Vacuum Method (2)—Official*

### 23.2

#### APPARATUS

*Vacuum oven*.—Connected with pump to maintain partial vacuum in oven with pressure equivalent to 25 mm or less of Hg and provided with thermometer passing into oven with bulb near samples. Connect an H<sub>2</sub>SO<sub>4</sub> gas-drying bottle to oven for admitting dry air to release vacuum.

### 23.3

#### DETERMINATION

(a) *Liquid eggs*.—Weigh accurately by difference by means of weighing buret ca 5 g of sample, 23.1(a) or (b), in a covered dish that previously has been dried at 98–100°, cooled in desiccator, and weighed soon after attaining room temp. Remove cover and drive off most of H<sub>2</sub>O by heating on steam bath. Replace cover loosely and complete drying in vacuum oven as directed under (b).

(b) *Dried eggs*.—Weigh ca 2 g of the sample, 23.1(c) or (d), in covered dish that

previously has been dried at 98–100°, cooled in desiccator, and weighed soon after attaining room temp. Loosen cover (do not remove) and heat at 98–100° to constant weight (ca 5 hours) in vacuum oven. Admit dry air into oven to bring to atmospheric pressure. Immediately tighten cover of dish, transfer to desiccator containing fresh efficient desiccant, and weigh soon after room temp. is attained. Report weight of egg residue as percentage total solids.

#### ORGANIC AND AMMONIACAL NITROGEN (5)—OFFICIAL

##### 23.4

###### PREPARATION OF SAMPLE

(a) *Liquid eggs*.—Weigh 2–3 g of well-mixed sample, 23.1(a) or (b), by difference into 500 ml Kjeldahl flask.

(b) *Dried eggs*.—Transfer ca 1 g of sample, 23.1(c) or (d), accurately weighed, to 500 ml Kjeldahl flask.

##### 23.5

###### DETERMINATION

Determine N as directed under 2.24, 2.25, or 2.26. (Complete digestion is accomplished most rapidly by Kjeldahl-Gunning-Arnold method, 2.26.) Distil the  $\text{NH}_3$  into 30–50 ml of 0.1 N standard acid.

#### WATER-SOLUBLE NITROGEN AND CRUDE ALBUMIN NITROGEN (4)—OFFICIAL FOR LIQUID EGGS, TENTATIVE FOR DRIED EGGS

##### 23.6

###### PREPARATION OF SOLUTION

(a) *Liquid eggs*.—Weigh accurately, by difference, into 250 ml volumetric flask containing 150 ml of  $\text{H}_2\text{O}$ , ca 10 g of well-mixed sample, 23.1(a) or (b), and mix gently. Add 5 ml of 0.01 N acetic acid for each gram of egg substance, fill to mark with  $\text{H}_2\text{O}$ , shake gently, and filter thru  $18\frac{1}{2}$  cm folded filter, covering filter with watch-glass during filtration. If filtrate is cloudy, allow filtration to continue until drops of filtrate become clear, change receiving container, return cloudy filtrate to filter, and proceed as directed under 23.7.

(b) *Dried eggs*.—From the sample, 23.1(c) or (d), transfer 1 g of whites, 3 g of whole eggs or 5 g of yolks into an 8 oz. nursing bottle, add 50 ml of petroleum benzene, mix gently, centrifuge, and decant solvent. Repeat treatment with petroleum benzene. Place bottle on its side, rolling it occasionally until residue is dry, and break up the dry residue with glass rod having flattened end. Add 100 ml of  $\text{H}_2\text{O}$  (slowly at first with gentle mixing until sample disintegrates), then add 5 ml of 0.01 N acetic acid for each gram of egg substance and sufficient  $\text{H}_2\text{O}$  to make total of exactly 200 ml of  $\text{H}_2\text{O}$  and acid. Mix gently and allow to stand 2 hours, continuing mixing at intervals. Centrifuge, filter, and proceed as directed under 23.7.

##### 23.7

###### DETERMINATION

(a) *Water-soluble nitrogen*.—Transfer 50 ml of clear prepared filtrate into 500 ml Kjeldahl flask, and determine N as directed under 2.26, using  $\text{HgO}$ . Calculate the N and report as percentage of water-soluble N.

(b) *Crude albumin nitrogen*.—Transfer 100 ml of clear prepared filtrate to 200 ml volumetric flask, add 15 ml of NaCl soln (28 g NaCl diluted to 300 ml), fill nearly to mark with alcohol, and mix. Cool to room temp., fill to mark with alcohol, shake, and allow to stand overnight. Filter, transfer 100 ml of filtrate to 500 ml Kjeldahl flask, and determine N as directed under 2.26, using  $\text{HgO}$ . Calculate percentage of N, subtract it from percentage of water-soluble N, and report difference as percentage of crude albumin N.

## FAT BY ACID HYDROLYSIS (5)—TENTATIVE

## 23.8

## PREPARATION OF SOLUTION

(a) *Liquid eggs*.—From the well-mixed sample, 23.1(a) or (b), weigh accurately by difference into Mojonnier fat extraction tube ca 2 g of yolks, or 3 g of whole eggs, or 5 g of whites. Add slowly with vigorous shaking 10 ml of HCl, set tube in water bath heated to 70°, bring to boiling, and continue heating at boiling for 30 min., shaking tube with care at 5 min. intervals. Remove tube from water bath, add H<sub>2</sub>O nearly to fill lower bulb of tube, and cool to room temp.

(b) *Dried eggs*.—Transfer 1 g of well-mixed sample to fat extraction tube; add slowly, washing down any egg particles adhering to sides of tube, 10 ml of HCl (4+1); and proceed as directed in (a).

## 23.9

## DETERMINATION

To the extraction tube containing the treated sample, 23.8, add 25 ml of ether, and mix. Add 25 ml of redistilled petroleum benzine (b.p. below 60°), mix, and allow to stand until solvent layer is clear. Proceed as directed in 20.16, beginning "Draw off as much as possible," but omitting filtration.

LIPOIDS AND LIPOID PHOSPHORIC ACID (P<sub>2</sub>O<sub>5</sub>) (6)—TENTATIVE

## 23.10

## REAGENTS

(a) *Mixed solvent*.—Equal volumes of CHCl<sub>3</sub> and absolute alcohol.

(b) *Alcoholic sodium hydroxide*.—Prepare a soln free from carbonates by dissolving 100 g of NaOH in 100 ml of H<sub>2</sub>O. Allow mixture to stand until clear, or filter thru hardened filter paper that has been soaked in alcohol (5 ml of the NaOH soln contains ca 4 g of NaOH). Dissolve 50 ml of this soln in 900 ml of alcohol and dilute with alcohol to 1 liter.

## 23.11

## PREPARATION OF SOLUTION

(a) *Liquid eggs*.—Weigh accurately by difference ca 4 g of the well-mixed sample, 23.1(a) or (b), into 100 ml flask, add very slowly (dropwise) from a pipet, 25 ml of the mixed solvent, shaking constantly until proteins become coagulated and then thoroly broken up. Add 60–65 ml more of solvent and allow to stand 1 hour, shaking at 5 min. intervals. Fill to mark with solvent, shake, and allow mixture to stand until clear.

(b) *Dried eggs*.—Transfer 2 g of the well-mixed sample, 23.1 (c) or (d), to 100 ml volumetric flask, add 85–90 ml of the mixed solvent, and allow to stand 1 hour, mixing at 5 min. intervals. Proceed as directed in (a).

## 23.12

## DETERMINATION

(a) *Lipoids*.—Transfer 50 ml aliquot to 150 ml beaker and evaporate extract to dryness on steam bath. (Electric fan or gentle blast of dry air may be used to hasten evaporation.) Place beaker in oven at 100° for 5–10 min. to remove any remaining moisture. Dissolve dry extract in 5–10 ml of CHCl<sub>3</sub>, and filter soln into weighed 100 ml Pyrex beaker thru pledget of cotton packed into stem of funnel, transferring all soluble extract from bottom and sides of beaker by means of CHCl<sub>3</sub>. Finally wash funnel and stem tip (filtrate should be clear). Evaporate the CHCl<sub>3</sub> on steam bath, and dry beaker and contents in oven at 100° to constant weight (ca 90 min.). Allow beaker to stand in air to constant weight (ca 30 min.), weigh, and report percentage of lipoids.



(b) *Lipoid phosphoric acid* ( $P_2O_5$ ).—Dissolve dried lipoids in 2–3 ml of  $CHCl_3$ , add 10–20 ml of the alcoholic NaOH soln, evaporate to dryness on steam bath, using care to avoid spattering, and place beaker in oven at  $100^\circ$  for 30 min. to remove any remaining moisture. Transfer beaker while hot to electric muffle heated to  $500^\circ$  (faint redness), and allow it to remain at this temp. 1 hour. Cool, add a few drops of  $H_2O$ , break up charge with glass rod having flattened end, cover beaker with watch-glass, add slowly 5 ml of  $HNO_3$  (1+3), mix, wash watch-glass, and filter, collecting filtrate in 300 or 500 ml Erlenmeyer flask. Thoroughly wash charred material and filter paper with  $H_2O$ .

In filtrate determine phosphoric acid ( $P_2O_5$ ) as directed under 2.12, using 20–50 ml of the molybdate soln. Report percentage of lipid  $P_2O_5$  in the eggs.

#### CHOLESTEROL (7)—OFFICIAL

##### 1. Separation of Unsaponifiable Matter

#### 23.13

##### REAGENTS

(a) *Concentrated potassium hydroxide soln.*—Dissolve 60 g of KOH in 40 ml of  $H_2O$ .

(b) *Dilute potassium hydroxide soln.*—Dissolve 10 g of KOH in 1 liter of  $H_2O$ .

(c) *Ether.*—Ethyl ether, U.S.P., free of peroxides by U.S.P. test applied immediately before use

(d) *Dried ether.*—Immediately before use shake peroxide-free ether U.S.P. with an excess of anhydrous  $CaCl_2$  and filter.

(e) *Anhydrous sodium sulfate.*—Powder to pass 60-mesh sieve.

#### 23.14

##### APPARATUS

(1) *Separators.*—One 250 ml and one 500 ml. *Wash separators free of grease.* They should be ether-tight with stopcocks lubricated only with  $H_2O$ .

(2) *Filtration bell jar.*—Large enough to accommodate a 300 ml Erlenmeyer flask, and provided with air-leak valve to control degree of vacuum.

(3) *Sintered glass filter.*—Jena 11G3 sintered-glass filter or equivalent fritted glass filter of like porosity.

#### 23.15

##### DETERMINATION

Weigh accurately into 125 ml Erlenmeyer flask ca 2.5 g of whole egg, 1.5 g of yolk, 1 g of dried whole egg, or 0.7 g of dried yolk, and add 10 ml of the conc. KOH soln. Cover with small watch-glass and heat 3 hours on steam bath, swirling occasionally to disintegrate any large lumps. Cool until just warm, add 30 ml of alcohol and swirl until all insoluble matter is *finely* dispersed. Add 50 ml of ether, mix thoroughly by swirling, and transfer to the 500 ml separator. Wash flask with 2 more 50 ml portions of ether and thoroughly mix the ether soln by swirling. Wash saponification flask with 100 ml of the dilute KOH soln, pour soln slowly into separator while gently swirling the liquid, and continue the gentle swirling for 10–15 seconds. Allow liquids to separate (ca 10 min.) and slowly draw off soap soln into the 250 ml separator, but do not draw off any small quantity of emulsion or insoluble matter at the interface. Rinse down sides of the 500 ml separator with 10 ml of the dilute KOH soln and draw this off into the smaller separator. Add 50 ml of ether to the smaller separator and shake vigorously. After the liquids have separated, discard lower layer. Add ether layer to soln in the large separator, rinsing the 250 ml separator with 10 ml of ether. Wash the ether soln as before with 100 ml of the dilute KOH

soln, still retaining any insoluble matter or emulsion in the separator. Add to the ether 20 ml of HCl (1+4), swirl, add 100 ml of H<sub>2</sub>O, and swirl again. Discard acid washings. Wash ether soln in same manner as before with two more successive 100 ml portions of the dilute KOH soln. Test a portion of the last washings for soap by acidifying with the HCl; the acidified washings should be clear or only faintly turbid. If necessary, repeat washing with the dilute KOH soln until acidified washings are clear. Wash ether soln successively by swirling with 50 ml of H<sub>2</sub>O, 50 ml of H<sub>2</sub>O containing 0.5 ml of 0.1 N HCl, and two more 50 ml portions of H<sub>2</sub>O. Finally, draw off as much of the H<sub>2</sub>O as possible without loss of ether soln. Filter ether soln into a dry 300 ml Erlenmeyer flask thru 15 g layer of Na<sub>2</sub>SO<sub>4</sub> on the sintered glass filter, using no suction for the first few ml and then gentle suction for the remainder. Rinse separator and filter successively with 10, 5, 5, 5 ml portions of ether. Rinse stem of filter with ether, add a porcelain chip to flask, and evaporate ether on steam bath. Dissolve residue in 20 ml of dried ether, and using 10, 5, and 5 ml portions of dried ether transfer ether soln thru small short-stemmed funnel to 50 ml Erlenmeyer flask (containing a porcelain chip). An approximate determination of unsaponifiable matter can be obtained by collecting ether solns in flask previously dried and weighed as follows: Dry flask containing the chip, and similar flask used as counterpoise, at 100–105° for 1 hour; remove from oven and place near balance for 30 min.; weigh flask, using counterpoise. Evaporate ether on steam bath. Wipe flask with clean towel, dry, and weigh with the counterpoise as before. Deduct from weight of unsaponifiable matter the blank obtained from reagents used, determined by same procedure.

## II. Determination of Cholesterol

### 23.16

#### REAGENTS

(a) *Ice*.—For four determinations have available ca 3 gallons of crushed ice.

(b) *Bromine soln*.—Weigh to 0.1 g a narrow-mouthed, glass-stoppered 25 ml flask containing 5 ml of CCl<sub>4</sub>. Add 4–5 g of Br, weigh again, and dilute with CCl<sub>4</sub> to calculated final concentration of  $0.22 \pm 0.02$  g of Br per ml. (This reagent should not be more than 2 days old.)

(c) *Acetic acid soln*.—Pipet 200 ml of acetic acid into a 250 ml glass-stoppered volumetric flask; dilute to mark with H<sub>2</sub>O, mix cautiously, dilute to mark, and mix again.

(d) *Asbestos*.—Prepare asbestos as directed in 34.38.

(e) *Sand*.—Pass clean sand thru 60-mesh sieve and treat with warm HCl until extracts are practically colorless. Wash, dry, and ignite.

(f) *Sodium hypochlorite soln*.—Dissolve 88 g of NaOH in 200 ml of H<sub>2</sub>O in wide-mouthed, 3 liter flask; add ca 1500 ml of crushed ice and pass in Cl until 71 g is absorbed; dilute to 2 liters and store in dark bottles in refrigerator. (Soln should be alkaline to phenolphthalein.) Before use, check concentration of available Cl as follows: Pipet 5 ml into flask containing soln of 2 g of KI in 100 ml of H<sub>2</sub>O, add 5 ml of 6 N HCl, and titrate with 0.1 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. The soln should be equivalent in available Cl to 0.95–1.05 N NaOCl.

(g) *Sodium formate soln*.—Prepare an aqueous soln of NaCHO<sub>2</sub> containing 0.5 g of the salt per ml.

(h) *Hydrochloric acid*.—Approximately 6 N; mix 520 ml of HCl (not less than 35% HCl by weight) with H<sub>2</sub>O and dilute to 1 liter.

(i) *Methyl red indicator*.—Dissolve 0.5 g of methyl red in 50 ml of alcohol, dilute to 100 ml with H<sub>2</sub>O, and filter. This soln must be free of insoluble matter, and it should be refiltered immediately before use if necessary.

(j) *Potassium iodide soln.*—20%. This soln must be colorless when acidified with HCl.

(k) *Starch soln.*—1% soln of soluble starch.

(l) *Sodium thiosulfate soln.*—0.02 *N*. Prepare from  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  and  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  containing 1% of amyl alcohol, 41.1(c). (Soln so prepared usually retains its titer for months.) Standardize with exactly 0.02 *N* soln of pure  $\text{KIO}_3$  as follows: To glass-stoppered 125 ml Erlenmeyer flask add 5 ml of the KI soln, 10 ml of  $\text{H}_2\text{O}$ , 1.5 g of  $\text{NaHCO}_3$ , and 5 ml of 6 *N* HCl. Mix, add 25–30 ml of the  $\text{KIO}_3$  soln, and titrate at once with the  $\text{Na}_2\text{S}_2\text{O}_3$  soln, using starch soln as indicator.

(m) *Potassium hydroxide soln.*—Dissolve 10 g of KOH in 10 ml of  $\text{H}_2\text{O}$ .

(n) *Ammonium molybdate soln.*—Dissolve 5 g of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  in 100 ml of  $\text{H}_2\text{O}$ .

### 23.17

#### APPARATUS

(a) *Ice bath.*—A container holding ca 4 liters and 10–15 cm deep filled with crushed ice.

(b) *Mohr pipets.*—One graduated to 0.01 ml; one graduated to 0.1 ml.

(c) *Filtration bell jar.*—Of sufficient size to accommodate a 300 ml Erlenmeyer flask, connected to a vacuum by a 2-way stopcock.

(d) *Device for filtering at 0°.*—A filter tube of the Knorr extraction tube style, ca 20 mm in diameter inside, with body ca 11 cm long and stem ca 10 cm long, provided with perforated disk of Ni or monel metal. Remove stem at apex from a 60° Bunsen funnel, 11 cm in diam. Enlarge opening at apex to ca 1 cm diam. by grinding or grating off the glass. Cut ca 1 cm from the end of a one-holed rubber stopper of a size that fits snugly in the opening of the funnel. Pass stem of filter tube thru the stopper in the funnel apex and then thru a stopper in the bell jar. Prepare in the filter tube a mat of the asbestos 6–8 mm thick and cover with ca 12 mm layer of the sand.

### 23.18

#### DETERMINATION

Pack the Br reagent and a 25 ml graduated cylinder in ice. Pack ice around the filter tubes, taking care none gets into the filters. Cool the acetic acid soln to ca –5° in an ice-NaCl mixture.

Wash down sides of the 50 ml Erlenmeyer flasks containing the unsaponifiable matter, while rotating them, with 2.0 ml of absolute ether delivered from a Mohr pipet; stopper with a cork, swirl until the porcelain chips are freed from sticking to the flasks, and pack flasks in ice bath up to the necks for at least 10 min. To one of the flasks add from a Mohr pipet 0.20 ml of the cold Br reagent, mix contents by swirling, stopper, and replace in ice bath. Start this procedure at 3 min. intervals with the other flasks (4 determinations can be made at one time if bell jars are available). After 10 min. add rapidly 15 ml of the acetic acid soln from the cold 25 ml cylinder, swirl liquid for 3 min. while holding in ice  $\text{H}_2\text{O}$ , and replace in ice bath for 10 min. With suction on, pour all mixture down a stirring rod into filter tube, leaving rod in filter. Wash down sides of flask with 5 ml of the cold acetic acid soln and replace in the ice bath. When liquid in filter just recedes below surface of sand, add the acetic acid from flask. Repeat washing in like manner with 5 ml of the acetic acid soln and suck filter free of excess liquid. Wash flask and filter with cold  $\text{H}_2\text{O}$ , filling filter tube about three times. Thoroughly drain flask and apply suction to filter until drops of  $\text{H}_2\text{O}$  cease to fall from stem. Remove ice pack from around filter tube and discard filtrate and washings. Place a 300 ml Erlenmeyer flask under filter so that stem projects well into neck of flask. Wash test tube and filter with 10 ml of

alcohol, 10, 5, and 5 ml portions of ether, and finally with 10 ml of alcohol, gently stirring sand with each portion of solvent and allowing mixture to stand ca 1 min. before applying suction. Wash stem of filter with a few ml of ether, add 1 ml of the KOH soln, mix, and wash down the sides of flask with 5 ml of ether. Evaporate the ether and alcohol completely on steam bath, finally using stream of clean air to remove last of alcohol vapors. Add 40 ml of hot H<sub>2</sub>O to residual alkaline liquid, mix, and neutralize alkali with 6 N HCl, using 1 drop of methyl red indicator. (This neutralization need be only approximate.) Add 10 g of NaCl, 3 g of NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O, and 20 ml of the NaOCl soln. Bring soln just to vigorous boiling, remove from heat, and add immediately, with care, 5 ml of the NaCHO<sub>2</sub> soln. Cool, and dilute to ca 150 ml with H<sub>2</sub>O. Add 5 ml of the KI soln, a drop or two of the ammonium molybdate soln, and 25 ml of the 6 N HCl. Titrate rapidly at once with the Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln, using starch soln as indicator. Correct titer for a blank determination on reagents, starting at point where the KOH soln is added to the alcohol-ether soln.

$$0.55 + 0.688 \times \text{ml of } 0.02 \text{ N Na}_2\text{S}_2\text{O}_3 = \text{cholesterol (mg)}.$$

#### TOTAL PHOSPHORIC ACID (P<sub>2</sub>O<sub>5</sub>) (g)—OFFICIAL

##### 23.19

##### PREPARATION OF SOLUTION

(a) *Liquid eggs*.—From the well-mixed sample, 23.1 (a) or (b), weigh accurately, by difference, into 250 ml low-form Pyrex beaker, ca 2 g of yolks, 4 g of whole eggs, or 10 g of whites. Add 20 ml of 10% Na<sub>2</sub>CO<sub>3</sub> soln and evaporate to dryness on electric hot plate or overnight at 100–105°. Transfer beaker while hot to electric muffle heated to 500° (faint redness), and allow it to remain at this temp. 1 hour. Cool, add a few drops of H<sub>2</sub>O, break up charge with glass rod having flattened end, cover beaker with watch-glass, add slowly while stirring 10 ml of HNO<sub>3</sub> (1+3), mix, wash watch-glass and filter, collecting filtrate in 300 or 500 ml Erlenmeyer flask. Thoroughly wash charred material and filter with H<sub>2</sub>O.

(b) *Dried eggs*.—Transfer 1 g of the well-mixed sample, 23.1 (c) or (d), to 150 ml low-form Pyrex beaker, add 20 ml of 10% Na<sub>2</sub>CO<sub>3</sub> soln, and proceed as directed under (a).

##### 23.20

##### DETERMINATION

In the prepared filtrate determine P<sub>2</sub>O<sub>5</sub> as directed under 2.12, using 40–50 ml of the molybdate soln. Report as total P<sub>2</sub>O<sub>5</sub>.

#### CHLORINE (g)

##### 23.21

##### Method I—Official

(a) *Liquid eggs* (in absence of added salt).—From the well-mixed sample, 23.1 (a) or (b), weigh accurately, by difference, into 150 ml low-form Pyrex beaker ca 4 g of yolks, 7 g of whole eggs, or 10 g of whites; add 20 ml of 10% Na<sub>2</sub>CO<sub>3</sub> soln, mix, and evaporate to dryness on electric hot plate or overnight at 100°. Transfer beaker while hot to electric muffle heated to 500° (faint redness), and allow to remain at that temp. 1 hour. Cool, add a few drops of H<sub>2</sub>O, and break up charge with glass rod. Add 50 ml of H<sub>2</sub>O, cover beaker with watch-glass, add slowly 20 ml of HNO<sub>3</sub> (1+3), wash watch-glass, mix, filter, and wash charred material and filter thoroughly with H<sub>2</sub>O. Proceed as directed in one of the following alternatives:

(1) To combined filtrate and washings add known volume of 0.1 N AgNO<sub>3</sub> in slight excess and proceed as directed in 12.44.

(2) Collect the filtrate and washings in 250 ml flask, keeping total volume of

filtrate to 180 ml or less. Add known volume of 0.1 *N* AgNO<sub>3</sub> in slight excess and make to volume. Filter, and using an aliquot of the filtrate, proceed as directed in 12.44, beginning "add 5 ml of the ferric indicator."

(b) *Liquid eggs* (in presence of added salt).—From the well-mixed sample, 23.1 (a) or (b), weigh 1–2 g accurately, by difference, into 150 ml low-form Pyrex beaker, and proceed as directed under (a).

(c) *Dried eggs*.—From the well-mixed sample, 23.1 (c) or (d), transfer to 150 ml low-form Pyrex beaker, 2 g of whole eggs or yolks, or 1 g of whites, and proceed as directed under (a).

### 23.22

#### *Method II (10)—Official*

From the well-mixed sample, 23.1 (a), (b), (c), or (d), weigh accurately, by difference, ca 4 g of yolks, 7 g of whole eggs, or 10 g of whites; or transfer 2 g of dried whole eggs or yolks, or 1 g of dried whites, into 300 ml Erlenmeyer flask. Add known volume of 0.1 *N* AgNO<sub>3</sub> in slight excess and 20 ml of HNO<sub>3</sub>, and place mixture on steam bath for 15–30 min. Add 15 ml of 5% KMnO<sub>4</sub> soln and allow mixture to stand 60–90 min. longer on steam bath. Cool to 25° or less, add 75 ml of H<sub>2</sub>O, 1 ml of nitrobenzene (or 1 ml for each 50 mg of Na Cl present), stopper flask, and shake vigorously to coagulate the precipitate. Add 5 ml of saturated ferric alum indicator and titrate with 0.1 *N* thiocyanate soln to end point that persists after soln has stood 15 min. (Titration should be made at 25° or below; soln is yellow green before end point and yellow orange at end point. At first permanent color change, note buret reading and time, stopper flask, shake vigorously, and allow to stand 15 min. If soln has faded, add thiocyanate soln in half-drop portions until end point color reappears (11). From number of ml of AgNO<sub>3</sub> used, calculate quantity of NaCl, after deducting blank run on reagents, using ca 0.25 g of sucrose instead of egg.

#### DEXTROSE AND SUCROSE (12)—OFFICIAL

### 23.23

#### PREPARATION OF SOLUTION

(a) *Liquid eggs*.—Weigh accurately, by difference, ca 25 g of well-mixed sample, 23.1 (a) or (b), into 250 ml volumetric flask containing 1 g of CaCO<sub>3</sub> and 50 ml of 5% NaCl. Add with continuous mixing 130 ml of alcohol. Allow to stand few min. for gas bubbles to rise to surface, cool to room temp., fill to mark with H<sub>2</sub>O, shake, and filter (18.5 cm folded filter). Transfer 150 ml of filtrate to 250 ml beaker, evaporate to 20–30 ml to remove alcohol, cool, and wash with H<sub>2</sub>O into 100 ml volumetric flask, holding volume to 80–90 ml; add dry powdered phosphotungstic acid in small quantities in slight excess to precipitate any protein, mix, let stand few min. for gas bubbles to rise to surface, fill to mark with H<sub>2</sub>O, shake, and filter. To filtrate, add in very small portions sufficient dry powdered KCl to precipitate any excess phosphotungstic acid, filter if necessary, and test filtrate for complete precipitation.

To correct for error due to volume occupied by precipitate in samples containing added sucrose, repeat determination, weighing same quantity of sample into 500 ml volumetric flask containing 1 g of CaCO<sub>3</sub> and 100 ml of 5% NaCl soln. Add, with continuous mixing, 260 ml of alcohol. Allow mixture to stand few minutes for gas bubbles to rise to surface, cool to room temp., fill to mark with H<sub>2</sub>O, shake, and filter thru 18.5 cm folded filter. Transfer 300 ml of filtrate to 400 ml beaker, evaporate to 20–30 ml, and proceed as directed above. To obtain amount of sucrose subtract percentage of sucrose obtained in 250 ml dilution determination from twice percentage obtained in 500 ml dilution determination.

(b) *Dried eggs*.—From the well-mixed sample, 23.1 (c) or (d), transfer to 250 ml

volumetric flask containing 1 g of  $\text{CaCO}_3$  and 50 ml of 5%  $\text{NaCl}$  soln 2.5 g of whites, or 10 g of yolks or whole eggs, and allow to stand 1 hour, mixing at 5 min. intervals. Add with continuous mixing 130 ml of alcohol, and proceed as directed under (a), beginning with the 3rd sentence.

**23.24****DETERMINATION**

*Reducing sugars direct.*—Transfer 25 ml of prepared filtrate to 400 ml beaker, and proceed as directed under 34.39. Report as percentage of dextrose.

*Reducing sugars invert.*—Transfer 50 ml of the prepared filtrate to 100 ml volumetric flask, and invert the sucrose as directed in 34.24(b) or (c). Neutralize with  $\text{NaOH}$  soln, cool to room temp., and fill to mark with  $\text{H}_2\text{O}$ . Transfer 50 ml (or less) to 400 ml beaker, and proceed as directed under 34.39. Deduct percentage of invert sugar obtained before inversion from that obtained after inversion, multiply difference by 0.95, and report as percentage of sucrose.

**GLYCEROL (15)***Qualitative Test—Tentative***23.25****REAGENT**

*Fuchsin-bisulfite soln.*—Dissolve 0.2 g of fuchsin in 120 ml of hot  $\text{H}_2\text{O}$ , cool, and add soln of 2 g of anhydrous  $\text{Na}_2\text{SO}_3$  in 20 ml of  $\text{H}_2\text{O}$ , then 2 ml of  $\text{HCl}$ . Dilute soln with  $\text{H}_2\text{O}$  to 200 ml and allow to stand 1 hour.

**23.26****DETECTION**

Add 20 ml of alcohol to ca 5 g of sample in Erlenmeyer or beaker flask, shake vigorously, and filter thru 12.5 cm fluted filter paper. Evaporate filtrate rapidly until no odor of alcohol is perceptible, cool, and add 3–4 drops of  $\text{H}_2\text{O}$  and then 10–15 ml of anhydrous ether. Mix solns carefully, allow to separate, and pour off as much as possible of ether layer, disregarding cloudiness in this layer. Shake well with two 10 ml portions of anhydrous ether, pouring off ether carefully in each case. (Volume of aqueous soln should not be less than 0.4–0.5 ml.) Evaporate remaining liquid on steam bath to 0.1–0.2 ml. Cool, and add 15 ml of mixture of equal volumes of absolute alcohol and  $\text{CHCl}_3$ . Cool, shake, and allow mixture to stand 5 min. to permit crystallization of sugar. Shake, and filter thru fluted filter paper into 6×1" test tube (hard glass). Evaporate filtrate rapidly (small flame in front of fan is convenient) until no odor of  $\text{CHCl}_3$  or alcohol is perceptible. Add several grams of powdered  $\text{K}_2\text{SO}_4$  and insert a stopper with a glass tube leading into 2 ml of  $\text{H}_2\text{O}$  in a test tube immersed in ice  $\text{H}_2\text{O}$ . Heat with small flame until frothing ceases and contents of tube are liquid. Remove receiver, add immediately 4–5 drops of the fuchsin-bisulfite reagent, and warm to room temp. In presence of glycerol a strong pink color (due to acrolein) develops within 1 min. and becomes a deep violet within 5 min.

*Quantitative Method—Tentative*

(Not applicable in presence of sugars)

**23.27****REAGENTS**

(a) *Mercuric nitrate soln.*—See 22.92.

(b) *Diphenylamine indicator*—See 33.75(d).

(c) *Phosphoric acid-sulfuric acid soln.*—Add 150 ml of  $\text{H}_2\text{SO}_4$  and 150 ml of sirupy phosphoric acid to 500 ml of  $\text{H}_2\text{O}$  and dilute with  $\text{H}_2\text{O}$  to 1 liter.

(d) *Potassium dichromate soln.*—See 33.75(a).

(e) *Ferrous ammonium sulfate soln.*—See 33.75(c).

(f) *Basic lead acetate soln.*—See 34.19(a).

(g) *Thymol blue indicator soln.*—Dissolve 0.1 g of thymol blue in 21.55 ml of 0.01 *N* NaOH and dilute to 250 ml with H<sub>2</sub>O.

## 23.28

## DETERMINATION

Weigh by difference ca 5 g of sample into 100 ml volumetric flask containing 50–75 ml of H<sub>2</sub>O, mix well, add 2 ml of the Hg(NO<sub>3</sub>)<sub>2</sub> soln, again mix well, and make up to mark with H<sub>2</sub>O. Mix, and transfer contents of flask to 8 oz. centrifuge bottle. Add 5 g of light magnesium carbonate, stopper, and shake vigorously for several min. Centrifuge 1–2 min., pour off supernatant liquid thru fluted filter, and transfer 75 ml to centrifuge bottle. Add 50 ml of the basic Pb acetate soln and then 50 ml of 2 *N* KOH, mix, and add a drop of the thymol blue indicator soln. If surface of liquid does not turn deep blue, add more alkali. Let stand 5–10 min., centrifuge until clear, and pour off liquid into 250 ml volumetric flask. Shake residue with 20 ml of H<sub>2</sub>O, centrifuge, and add washings to flask. Add 2 drops of thymol blue to soln and add H<sub>2</sub>SO<sub>4</sub> (1+1) until distinct pink color is obtained. Make up to mark with H<sub>2</sub>O, mix, and filter thru dry filter paper. Transfer 200 ml of filtrate to 400 ml beaker, evaporate rapidly to 75 ml, and finish evaporation on water bath or slow hot plate to 35–40 ml. Transfer liquid to 50 ml volumetric flask and make up to volume with H<sub>2</sub>O. Transfer 25 ml to 250 ml volumetric flask, and add 20 ml of the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> soln and 25 ml of H<sub>2</sub>SO<sub>4</sub>. Run a blank, using 20 ml of the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> soln and 25 ml of H<sub>2</sub>O. Heat in *boiling* water bath exactly 20 min. Cool, dilute to volume, mix, and transfer some of soln to buret. Pipet 20 ml of the Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> soln into beaker, add 100 ml of H<sub>2</sub>O, 15 ml of the H<sub>3</sub>PO<sub>4</sub>-H<sub>2</sub>SO<sub>4</sub> soln and just 3 drops of the diphenylamine soln, and titrate with the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> soln. When the green color has changed to blue-gray, add the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> soln slowly, swirling after each drop. End point is reached when addition of 1 drop of the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> soln changes color to deep violet. Subtract 0.05 ml from reading to correct for oxidation of indicator.

Percentage glycerol =  $100(a-b)/W$ , in which  $a$  = ml of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> titrated in unknown soln;  $b$  = ml of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> titrated in blank soln; and  $W$  = weight of sample in grams.

## ACIDITY OF ETHER EXTRACT

(Not applicable to egg white)

*Method I (14)—Official*

## 23.29

## REAGENTS

(a) *Benzene.*—Use best available quality of benzene. If it is not neutral, titrate 50 ml with the 0.05 *N* Na ethylate, reagent (b), and correct subsequent results accordingly.

(b) *Sodium ethylate.*—0.05 *N*. Dissolve a piece of metallic Na, ca 1 ml in volume, in 800 ml of absolute alcohol. Titrate 10 ml of 0.1 *N* HCl with this soln and add calculated volume of absolute alcohol to make soln 0.05 *N*. Ascertain normality factor by titration against 0.1 *N* HCl on day soln is used.

## 23.30

## DETERMINATION

(a) *Dried eggs.*—Weigh to nearest milligram ca 2 g of powdered sample, 23.1(c) or (d), in tared Al dish, ca 63 mm in diam., and dry at 55° under pressure not exceed-

ing 125 mm of Hg. Weigh at end of 2 hours and continue drying for half-hour periods to constant weight. Carefully transfer dried residue to 12.5 cm hardened filter paper, fold paper once, place it on a 15 cm qualitative filter paper, and roll papers and contents into cylinder that will fit snugly into extraction tube, folding in one end of cylinder to prevent loss of material. Extract with anhydrous ether, preferably in Knorr apparatus. (An asbestos plug is not needed in extraction tube, and if extractor is working rapidly, 3 hours is sufficient for proper extraction.) Evaporate ether from extraction flask, dry extract 1 hour at 55° under pressure not exceeding 125 mm, and weigh to nearest milligram. Dissolve extract in 50 ml of benzene, add 3 to 4 drops of phenolphthalein indicator, and titrate with the Na ethylate soln. The end point is reached when yellow color changes to orange. Report as ml of 0.05 *N* Na ethylate required per g of ether extract.

(b) *Liquid eggs*.—Weigh to nearest milligram in tared Pb dish ca 5 g of sample, 23.1(a) or (b), and dry as directed under (a). Weigh after ca 5 hours and continue drying for 1 hour periods to constant weight. To prepare dried residue for extraction with anhydrous ether, place dish upon 12.5 cm hardened filter paper, cut sides of dish thru at 4 equidistant points, and flatten down. Place another similar filter paper on top of dish and its contents, roll papers and dish into cylinder, and proceed as directed under (a).

#### *Method II—Rapid Method—Tentative*

### 23.31

#### REAGENT

*Salt soln.*—Dissolve 10 g of NaCl in ca 50 ml of H<sub>2</sub>O, add 30 ml of alcohol, and dilute to 100 ml with H<sub>2</sub>O.

### 23.32

#### DETERMINATION

(a) *Dried eggs (15)*.—Weigh 2 g of dried eggs into small-lipped Erlenmeyer flask, add ca 30 ml of ether, and mix well. After ether layer clears, decant thru small filter paper into weighed flask. Repeat extraction with ether 2–3 times, using ca 15 ml each time, and finally wash filter paper and funnel with ether. Evaporate the ether on steam bath and dry flask containing extract 30 min. at 100°. Cool, weigh, dissolve in 30 ml of benzene, and proceed as directed in 23.30(a), beginning “add 3 to 4 drops of phenolphthalein indicator.”

(b) *Liquid eggs (16)*.—Weigh 10 g of mixed whole liquid egg or 5 g of liquid yolks and transfer to suitable centrifuge bottle with 40 ml of the NaCl soln. Shake gently until egg is thoroly mixed with the NaCl soln. Add 50 ml of ether followed by 50 ml of petroleum benzine, stopper bottle, and shake gently but thoroly until lipoids are extracted. (Solvent layer is yellow when lipoids are extracted. If the solvent layer is not yellow, shake more vigorously.) Centrifuge to separate liquids. If bad emulsion has formed, add 10 ml of alcohol, shake gently, and centrifuge again. Remove ether layers by carefully pouring off or blowing off with wash bottle arrangement, or by any other method that will separate the solvent layer. Repeat extraction, using 30 ml each of ether and petroleum benzine, and add to first solvent shakeout. If only acidity of ether extract is to be determined, enough lipoids will be obtained by two extractions for titration purposes. If complete extraction is desired, repeat shaking with ca 50 ml of mixed ethers, centrifuging, etc., until ether layer is colorless after separation.

Evaporate mixture of solvents from first two extracts in suitable dish on steam bath. When mixture is removed, add 5 ml of absolute alcohol and evaporate again on steam bath to aid in removal of moisture. Dissolve residual extract in small quantity



of  $\text{CHCl}_3$ , filter into tared beaker, and wash dish and filter with  $\text{CHCl}_3$ . Evaporate off the  $\text{CHCl}_3$  on steam bath and continue heating few min. after  $\text{CHCl}_3$  is removed. Dry beaker with towel. Cool, and weigh.

Proceed as directed under 23.30(a), beginning "Dissolve extract in 50 ml of ben-

#### AMMONIA NITROGEN (17)—TENTATIVE

(For liquid eggs)

##### 23.33

##### APPARATUS

Apparatus consists of a train, items (a), (b), (c), and (d), each provided with a two-holed rubber stopper so connected as to permit proper passage of air, which is supplied by a pump with pressure of 10 lbs./sq. in. Compensate for pulsations of pump to assure delivery of steady pressure by placing tank of sufficient size between pump and bottle (a). Suction may be used to draw air thru train but pressure is preferable.

(a) *Wash bottle*.—Contains  $\text{H}_2\text{SO}_4$  (ca 35%) for removal of ammonia from the air supply. The inlet tube is provided with stopcock to regulate the air supply.

(b) *Trap*.—To prevent mechanical transfer of  $\text{H}_2\text{SO}_4$  into (c).

(c) *Aerating cylinder*.—About 50 mm in diam. and 350 mm high. The inlet tube extends to within  $\frac{1}{2}$ " of bottom. The outlet tube is provided with trap containing cotton or glass wool to prevent liquid from being carried over.

(d) *Bottle*.—Wide-mouthed, 8 oz. Inlet tube terminates in small bulb punctured with a few small holes to expedite  $\text{NH}_3$  absorption.

##### 23.34

##### DETERMINATION

Weigh ca 25 g of sample, 23.1(a) or (b), in convenient container. Pour as much as possible of this material into aeration cylinder (c) and transfer remainder by means of four 25 ml portions of  $\text{NH}_3$ -free  $\text{H}_2\text{O}$ , stirring each time with policeman to remove any egg adhering to sides of weighing vessel. Add 75 ml of alcohol, mix well, and let stand for 15 min. Add ca 1 g of NaF, 5 ml of 20%  $\text{Na}_2\text{CO}_3$  soln, and 1 ml of kerosene, or if necessary let stand overnight before adding the  $\text{Na}_2\text{CO}_3$ .

Connect train and aerate thru 10 ml of 0.02 N  $\text{H}_2\text{SO}_4$  (if sample has bad odor, more acid may be required), 2 drops of methyl red indicator (saturated soln in alcohol), and ca 75 ml of  $\text{NH}_3$ -free  $\text{H}_2\text{O}$  in the receiving bottle (d).

Use as rapid a current of air as possible without splashing the egg soln into the trap following cylinder (c). Determine time to aerate as follows: In a duplicate train measure 20 ml of  $\text{NH}_4\text{Cl}$  or  $(\text{NH}_4)_2\text{SO}_4$  soln (ca 5% stronger than 0.02 N) into aeration cylinder (c) and 20 ml of 0.02 N  $\text{H}_2\text{SO}_4$ , 75 ml of  $\text{H}_2\text{O}$ , and several drops of methyl red indicator into receiving bottle (d). Aerate until soln in receiving bottle changes color, noting time required. The sample should be aerated 30 min. longer than this time.

Titrate excess of acid in cylinder (d) with 0.02 N NaOH (free from  $\text{CO}_2$ ). Express results as mg of ammonia N/100 g of sample. Correct results for a blank determination on apparatus and reagents.

#### LACTIC ACID (18)—OFFICIAL

##### 23.35

##### PREPARATION OF SOLUTION

(a) *Liquid or frozen eggs*.—Transfer 40 g of sample to tared 300 ml Erlenmeyer flask, add ca 75 ml of  $\text{H}_2\text{O}$ , and shake thoroly. Add 15 ml of normal  $\text{H}_2\text{SO}_4$  and 25 ml

of 20% phosphotungstic acid soln, make to 200 g with  $H_2O$ , shake ca 1 min., and filter thru folded filter paper.

(b) *Dried eggs*.—Mix 10 g of sample and 100 ml of  $H_2O$  into uniform paste with aid of stirring rod and add with constant stirring 10 ml of normal  $H_2SO_4$ , followed by 15 ml of 20% phosphotungstic acid soln. Transfer mixture with  $H_2O$  to tared 300 ml Erlenmeyer flask, make to 200 g with  $H_2O$ , shake ca 1 min., and filter thru folded filter paper.

Weigh 100 g of filtrate obtained above into 250 ml beaker and evaporate to ca 25 ml. Transfer material into the liquid extractor with 25 ml of  $H_2O$  and proceed as directed under 22.8. Report lactic acid in terms of mg/100 g, making no correction for insoluble solids in portion taken for analysis.

#### VOLATILE FATTY ACIDS (18)—OFFICIAL

##### 23.36

##### PREPARATION OF SOLUTION

(a) *Liquid or frozen eggs*.—Weigh 80 g into tared 500 ml Erlenmeyer flask, add ca 150 ml of  $H_2O$ , and shake vigorously.

(b) *Dried eggs*.—Weigh 25 g into 250 ml beaker, and with a heavy stirring rod make into smooth paste with  $H_2O$ . Transfer contents of beaker to tared 500 ml Erlenmeyer flask, using a total of ca 200 ml of  $H_2O$ .

To samples add 25 ml of normal  $H_2SO_4$  and shake ca 1 min. Add 40 ml of a 20% phosphotungstic acid soln, make to 350 g with  $H_2O$ , and shake for 1 min. Filter thru a 24 cm folded filter paper. Weigh 150 g of the filtrate into 300 ml Erlenmeyer flask, add an excess of  $Ag_2SO_4$  (ca  $\frac{1}{2}$  g), and heat to boiling on hot plate under water-cooled reflux condenser. Cool flask with running  $H_2O$  while still connected with condenser. Transfer contents of flask to 200 ml volumetric flask with  $H_2O$ , make to mark, shake, and filter thru folded filter paper. Test filtrate for complete removal of chlorides with few crystals of  $Ag_2SO_4$ . If all the chlorides have not been removed, add more  $Ag_2SO_4$  to soln, shake for several minutes, and again filter, pouring back until bright. (In rare cases when it is not possible to get a bright filtrate, the addition of Filter-Eel and refiltration will prove helpful.)

##### 23.37

##### DETERMINATION

Proceed as directed under 24.11. In calculation of acids, divide quantity of formic or acetic acid, determined as being present in distillation flask, by 8.04 for dried eggs and by 25.71 for liquid eggs. Multiply by 100 to obtain mg of the acid in 100 g of sample.

##### 23.38 EXTRACTION AND IDENTIFICATION OF ADDED COLOR—TENTATIVE.—See 20.124.

#### SELECTED REFERENCES

- (1) J. Assoc. Official Agr. Chem., 8, 599 (1925); 9, 56 (1926).
- (2) Ibid., 8, 600 (1925); 9, 56, 354 (1926); 14, 85, 395 (1931).
- (3) Ibid., 8, 601 (1925); 9, 57 (1926).
- (4) Ibid., 15, 75, 344 (1932); 16, 74 (1933); 18, 80 (1935).
- (5) Ibid., 8, 601 (1925); 9, 58 (1926); 16, 73, 298 (1933).
- (6) Ibid., 7, 91 (1923); 8, 602 (1925); 9, 58 (1926); 16, 73, 298 (1933).
- (7) Ibid., 24, 119 (1941); 25, 87, 365 (1942); 27, 88 (1943).
- (8) Ibid., 14, 85, 416 (1931); 16, 73, 298 (1933); 18, 80 (1935).
- (9) Ibid., 16, 298 (1933); 18, 80 (1935); 22, 77, 302 (1939).
- (10) Ibid., 26, 352 (1943); 27, 88 (1944); 28, 79 (1945).
- (11) Ind. Eng. Chem., Anal. Ed., 7, 38 (1935).

- (12) J. Assoc. Official Agr. Chem. **14**, 397 (1931); **16**, 74, 305 (1933); **22**, 77, 302 (1939).
- (13) Ibid., **15**, 331 (1922); **16**, 74, 293 (1933).
- (14) Ibid., **10**, 50, 411 (1927).
- (15) Ibid., **15**, 341 (1932); **25**, 88 (1942).
- (16) Ibid., **21**, 85, 179 (1938).
- (17) Ibid., **19**, 93, 201 (1936).
- (18) Ibid., **27**, 204 (1944); **28**, 79 (1945).

## 24. FISH AND OTHER MARINE PRODUCTS

### 24.1

#### APPARATUS—TENTATIVE

(a) *Funnel*.—Made of metal, tin plated or brass, 8–10" in diameter at top, with stem 3" in diameter and ca 3" long. (b) *Measures*.—Straight-sided cylindrical, made of metal, holding exactly 1 gallon and 1 quart, respectively, and having smooth rims. The plane of the rim should be level when the measure is standing on a level surface. The diameter of top of gallon measure should not be more than 5½" or less than 4½", and that of quart measure not greater than 3½" or less than 3¼". Carefully calibrate these measures with standard glass measures. For estimating volumes short of level-full, use a graduated mechanic's depth gage to measure distance from rim to surface of contents. These depth gage readings may be tabulated against the volumes or percentage shortages as desired for each measuring vessel. (c) *Skimmer*.—A flat-bottomed pan or tray, with 2" sides. The bottom is perforated with holes ¼" in diameter and centers 1½" apart in a square pattern. Area of bottom of tray should be such that oysters are not over one layer deep (150 sq. in. for 1 quart of oysters). The skimmer should be supported over a solid tray slightly larger to receive the liquid. (d) *Meat chopper*.—With plate having holes ½" in diameter. It should not leak around handle end. (e) *Table fork*.—With sharp-edged tines ½" apart (stainless steel). (f) *Small sharp knife*. (g) *Malted milk stirrer*.—Electric.

### 24.2 PRELIMINARY TREATMENT AND PREPARATION OF SAMPLE—TENTATIVE

To prevent loss of H<sub>2</sub>O during preparation and subsequent handling, do not use small samples. Keep ground material in glass or similar containers provided with air- and water-tight covers. Prepare samples for analysis in following manner:

(a) *Fresh fish*.—Clean and prepare in usual manner. In case of small fish (6" long or less), remove one longitudinal half from each of 5–10 fish. In case of large fish, cut from each of at least 3 fish, 3 transverse slices, 1" thick; one slice from immediately back of pectoral fins, one slice halfway between first slice and vent, and one slice immediately back of vent. Separate any bones that may be present as completely as possible from pieces selected, leaving skin intact so far as possible, since in many fish large quantities of fat are stored directly beneath the skin; pass rapidly thru food chopper 3 times, thoroly mixing after each grinding; and begin all determinations as soon as practicable. If any delay occurs, chill sample to inhibit decomposition.

(b) *Canned salmon and similar types of canned fish*.—Pass entire contents of tin thru meat chopper 3 times, *thoroly mixing each time*.

(c) *Canned fish packed in oil*.—Drain fish on ¼"-mesh sieve (or larger mesh that will retain all the meat) for 5 min. Return to sieve any meat particles passing thru. Prepare solid portion as directed in (b). (Oil and brine may be separated and analyzed if desired.)

(d) *Shellfish other than oysters and scallops*.—If sample is received in the shell, separate edible portions in customary manner. Prepare edible portion for analysis as directed in (b).

(e) *Shell oysters and scallops*.—Wash the shells in potable H<sub>2</sub>O to remove all loose silt and dirt, and drain well. Shuck into clean dry container enough oysters to yield at least 1 pint of drained meats. Transfer oysters to skimmer, rinse lightly with spray of H<sub>2</sub>O to remove silt particles, and pick out pieces of shell; drain 1 min. on skimmer

and remove to glass fruit jar or other suitable container. Proceed as directed in (f), beginning "Grind the meats . . ."

(f) *Shucked oysters*.—"Fluff" the entire contents of the commercial container, or container in which sample is received (1 gallon or less is size) by pouring into standard measuring vessel thru distance of at least 1 foot, then likewise back into container, and again into measuring vessel. Measure head space with the depth gage, and determine volume. Transfer oysters to skimmer, drain 1 min., return meats to measuring vessel, and measure head space. Loss in volume is the free liquid. If less than 10%, again mix in with the meats. If the free liquid is more than 10% of total volume, allow it to set in tall container, remove scum from top, pour off clear liquid from shell and other sediment (do not filter), and analyze separately.

Grind meats or mixed meats and liquid in the meat chopper, remove any muscle that is retained inside chopper, and comminute with the fork and knife. (For good checks it is important to cut these pieces into small bits.) Mix all together in tin can of suitable size and stir with electric stirrer for 5 min., keeping entire contents of container in motion. If the impeller spins in one spot without moving entire contents, raise or lower tin container. Keep prepared samples under refrigeration between 1° and 10°.

NOTE: The malted milk stirrer cannot be used successfully with ground scallop meats; they should be mixed thoroly by stirring with a spatula.

(g) *Fish packed wet in salt and brine*.—Drain off brine and rinse off adhering salt crystals with saturated salt soln. Drain again for 2 min. and proceed as directed in (a); in case of sardine or anchovy types of small fish, as in (b).

(h) *Dried smoked or dried salt fish*.—Cut large samples into small pieces, mix, and quarter down to ca  $\frac{1}{4}$  lb. Cut, shred, grind, or otherwise comminute the  $\frac{1}{4}$  lb. sample as finely as possible so that reasonably representative samples may be weighed for analysis after being thoroly mixed. (Duplicate or triplicate determinations may be necessary to establish uniformity of sample.)

## 24.3

### TOTAL SOLIDS—TENTATIVE

(Oysters and scallops only)

Make duplicate determinations. Weigh quickly 10 g of the meats, liquid, or mixed meats and liquid in a flat-bottomed metal dish ca 9 cm in diameter. Spread sample evenly over bottom of dish. Evaporate just to dryness on steam bath and dry for 4 hours in water oven at temp. of 98–100°. Cool in desiccator and weigh promptly.

## 24.4

### ASH—OFFICIAL

Dry sample representing ca 2 g of dry material and proceed as directed under 34.9 or 34.10, at temp. not exceeding 550°. If material contains large quantity of fat, make preliminary ashing at sufficiently low temp. to allow smoking off of fat without burning.

### SALT (CHLORINE AS SODIUM CHLORIDE)

#### I. Open Carius Method—Official

## 24.5

### REAGENTS

(a) *Silver nitrate soln*.—0.1 *N*. Standardize against 0.1 *N* NaCl soln containing 5.846 g of pure dry NaCl per liter.

(b) *Ammonium thiocyanate soln*.—Standardize against the 0.1 *N* AgNO<sub>3</sub> soln.

(c) *Ferric indicator*.—A saturated soln of ferric ammonium alum.

## 24.6

## DETERMINATION

In case of oysters and scallops, put 10 g of the meats, liquid, or mixed meats and liquid into a 250 ml Erlenmeyer flask or beaker. In case of other fish products, use suitably sized sample, depending on salt content. Add known volume of the  $\text{AgNO}_3$  soln, more than sufficient to precipitate all the Cl as  $\text{AgCl}$ , and then add 20 ml of  $\text{HNO}_3$ . Boil gently on hot plate or sand bath until all solid matter except the  $\text{AgCl}$  is dissolved (usually 15 min.). Cool, add 50 ml of  $\text{H}_2\text{O}$  and 5 ml of indicator, and titrate excess Ag with the thiocyanate until permanent light brown color appears. Subtract ml of 0.1  $N$  thiocyanate used from ml of 0.1  $N$   $\text{AgNO}_3$  added and calculate quantity of Cl as NaCl. With 10 g sample each ml of 0.1  $N$   $\text{AgNO}_3$  = 0.058% NaCl.

## 24.7

*II. With Calcium Acetate as Fixative—Tentative*

In case of oysters and scallops, to 10 g of ground meats or liquid in Pt dish, add and thoroly incorporate 10 ml of 10% Ca acetate soln. In case of other fish products, use suitably sized sample, depending on salt content. Dry on steam bath, and ash in muffle at lowest visible red heat ( $550^\circ$ ). (Complete ashing is not necessary.) Dissolve ash in the Pt dish in 25 ml of  $\text{HNO}_3$  (1+3). Add to  $\text{HNO}_3$  soln a known volume of 0.1  $N$   $\text{AgNO}_3$  soln, more than sufficient to precipitate the chlorides as  $\text{AgCl}$ . Heat to boiling, cool, and add 5 ml of the  $\text{FeNH}_4(\text{SO}_4)_2$  indicator and titrate excess Ag with the thiocyanate until permanent light brown color appears. From ml of 0.1  $N$   $\text{AgNO}_3$  used, calculate quantity of Cl as NaCl. With 10 g sample, each ml of 0.1  $N$   $\text{AgNO}_3$  soln = 0.058% NaCl. Make a correction for Cl in the 10 ml of 10% Ca acetate soln if it is not free from chlorides.

## 24.8

## TOTAL NITROGEN (1)—OFFICIAL.—See 2.24, 2.25, or 2.26

## VOLATILE FATTY ACIDS (2)—OFFICIAL

## 24.9

## PREPARATION OF SOLUTION

(Thruout determination all  $\text{H}_2\text{O}$  should be boiled and protected from  $\text{CO}_2$ .)

Comminute sample (include entire contents with canned products) and mix until uniform (passing 3 times thru meat chopper and mixing after each grinding is satisfactory). Weigh 50 g of the comminuted material into tared 500 ml wide-mouthed Erlenmeyer flask. Add ca 150 ml of  $\text{H}_2\text{O}$ , stopper flask, and shake vigorously ca 1 min. to effect thoro suspension of material. Add 25 ml of normal  $\text{H}_2\text{SO}_4$ , mix, precipitate proteins with 20% phosphotungstic acid soln (25–40 ml), make to 300 g with  $\text{H}_2\text{O}$ , shake vigorously ca 1 min., and filter thru folded filter paper. Weigh 150 g of filtrate into 200 ml volumetric flask, add 3 drops of phenol red indicator, and neutralize with saturated  $\text{Ba}(\text{OH})_2$  soln. Add sufficient  $\text{AgClO}_4$  soln (1+1) to precipitate chlorides (5 ml is usually sufficient), make to mark, shake, and filter thru folded filter paper.

## 24.10

## STANDARDIZATION OF DISTILLATION APPARATUS

So place apparatus in laboratory that it is free from drafts and sudden changes in temp. Make a mark on the 3 liter boiler flask at 1500 ml level, fill to mark with boiled  $\text{H}_2\text{O}$ , heat to boiling, and boil for several minutes before starting distillation. Transfer 50 ml of ca 0.1  $N$  formic acid to distillation flask, and add 1 drop of  $\text{H}_2\text{SO}_4$  (1+1) and 100 ml of  $\text{H}_2\text{O}$ . Connect condenser, insert steam inlet tube into distillation flask, and bring contents of flask to incipient boiling by means of a burner. Connect steam inlet tube with steam supply of boiler and steam distil. So regulate rate of evolution

of steam and height of small flame of burner under distillation flask that volume of liquid in distillation flask is kept constant at 150 ml and distillate is collected at rate of 200 ml/hour. (Period of collection may vary  $\pm 1$  min. for 50 ml distillate and  $\pm 2$  min. for 200 ml distillate. The 150 ml volume in distillation flask should remain constant within  $\pm 5$  ml. Boiling may be stopped to permit test of constancy of 150 ml volume by momentarily interrupting steam supply. A few trials with  $H_2O$  will show conditions necessary to maintain constant volume in distillation flask and constant

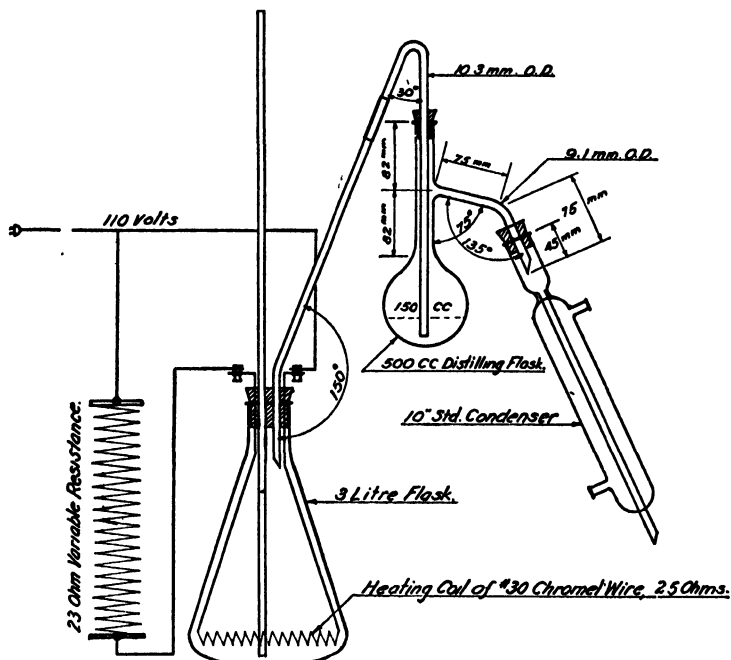


FIG. 37.—STEAM DISTILLATION ASSEMBLY CONSISTING OF BOILER FLASK (3 LITER) GIVING STEAM AT UNIFORM RATE SO AS TO PRODUCE CONSTANT RATE OF DISTILLATION, DISTILLATION FLASK, CONDENSER, AND 50 ML AND 200 ML VOLUMETRIC FLASKS AS RECEIVERS

rate of distillation.) Collect in volumetric flasks a 50 ml portion of distillate, followed immediately by a 200 ml portion. Transfer contents of 50 ml flask to 125 ml Erlenmeyer flask with ca 10 ml of  $H_2O$ , and contents of 200 ml flask to 300 ml Erlenmeyer flask with ca 20 ml of  $H_2O$ , and titrate each to phenolphthalein end point with 0.1  $N$  alkali. Determine blank on 50 ml and 200 ml distillates from 150 ml of  $H_2O$  and 1 drop of  $H_2SO_4$  (1+1) in distillation flask. Subtract respective blanks and calculate, in terms of percentage, the fractions of total formic acid originally present, which were carried over into 50 ml and 200 ml distillates. Designate as  $TF_1$  and  $TF_2$ , respectively. (Distillation fractions for standardization made in triplicate should check within range of 1%.)

Repeat standardization, using 0.1  $N$  acetic acid. Calculate percentage fractions and designate that of 50 ml distillate as  $TA_1$  and that of 200 ml distillate as  $TA_2$ . Calculate the ratio  $TA_2/TA_1$  and designate as  $CA$ .

## 24.11

## DETERMINATION

(a) *Buffer soln.*—Dissolve 12.404 g of  $\text{H}_3\text{BO}_3$  and 14.912 g of KCl in  $\text{H}_2\text{O}$  and dilute to 1 liter. Mix 75 ml with 36 ml of 0.1 N NaOH (or its equivalent), and make to 300 ml with  $\text{H}_2\text{O}$ . (Soln has pH of 8.6.) Transfer 60 ml of soln to thoroly clean 125 ml Erlenmeyer flask, and 220 ml to 300 ml Erlenmeyer flask; add 3 and 5 drops of phenolphthalein, respectively, to flasks, stopper tightly with rubber stoppers, and swirl.

(b) *Distillation and titration.*—Pipet 150 ml of filtrate, 24.9, into distillation flask of apparatus, and make acid to congo red paper with  $\text{H}_2\text{SO}_4$  (1+1). Steam distil as specified under 24.10, and collect one 50 ml portion followed by one 200 ml portion of distillate. Transfer 50 ml distillate to 125 ml Erlenmeyer flask with ca 10 ml of  $\text{H}_2\text{O}$ , and 200 ml distillate to 300 ml Erlenmeyer flask with ca 20 ml of  $\text{H}_2\text{O}$ . Titrate 50 ml distillate with 0.01 N  $\text{Ba}(\text{OH})_2$  until color matches that of prepared 60 ml buffer soln, and titrate 200 ml distillate to match 220 ml buffer soln. (Alkali should be kept in heavily paraffined bottles. Titration outfit described in *J. Assoc. Official Agr. Chem.*, 21, 686, and readily constructed from material in laboratory, has been found very convenient for this purpose.) Indicator color should persist ca 10 seconds. Determine blank on 50 ml and 200 ml distillates from 150 ml of  $\text{H}_2\text{O}$  and 1 drop of  $\text{H}_2\text{SO}_4$  (1+1) in distillation flask, and correct titrations for respective blanks.

(c) *Determination of formic acid.*—Combine 50 ml and 200 ml portions of titrated distillate obtained in (b), add 2 drops of saturated  $\text{Ba}(\text{OH})_2$  soln, and evaporate to dryness on steam bath. Add ca 5 ml of  $\text{H}_2\text{O}$  to residue and 1 ml more of normal HCl than is necessary to liberate volatile acids. Filter thru small paper into 125 ml Erlenmeyer flask with ground joint, and wash paper with  $\text{H}_2\text{O}$  in such manner that total filtrate will equal 30–40 ml. Add 10 ml of Na acetate-NaCl mixture (25 g of  $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$  plus 12 g of NaCl made to 500 ml) and 10 ml of 5%  $\text{HgCl}_2$  soln. Connect flask with ground joint air condenser, and place on steam bath for 2.5 hours. Transfer precipitate of  $\text{HgCl}_2$  to previously weighed A.C.S. type microfunnel, with coarse glass disk provided with mat of asbestos ca 2 mm thick, with aid of suction thru glass siphon attached to funnel by rubber stopper. Rinse flask with  $\text{H}_2\text{O}$  followed by alcohol. Dry 0.5 hour at  $100^\circ$ . Cool and weigh. Weigh funnel with another funnel, prepared with asbestos and treated in same manner as one containing precipitate, as counterpoise. Weight of calomel in  $\text{mg} \times 0.0975 = \text{mg}$  of formic acid in distillates. To calculate total formic acid originally present in aliquot of sample in distillation flask before distillation, divide result by sum of  $\text{TF}_1$  and  $\text{TF}_2$ , as determined in 24.10, and multiply by 5.33 to obtain  $\text{mg}/100 \text{ g}$  of formic acid in sample being analyzed.

(d) *Computation of acetic acid.*—

(1) *Formic acid present.*—Convert  $\text{mg}$  of formic acid in aliquot of sample in distillation flask before distillation, as calculated in (c), to ml of 0.01 N formic acid by dividing by 0.46. Multiply result by  $\text{TF}_1$  to obtain ml of 0.01 N alkali required to neutralize formic acid in 50 ml distillate, and subtract from titration (corrected for its blank) of 50 ml distillate obtained under (b). Designate as  $t_1$ , which result represents titration due to acetic acid in 50 ml distillate. Repeat calculation with 200 ml distillate, using  $\text{TF}_2$  as multiplier, and designate as  $t_2$ . Calculate ratio  $t_2/t_1$  and compare with CA as determined under 24.10. If comparison shows agreement ( $\pm 0.2$ ), acetic and formic are the only acids present. Sum of titrations  $t_1$  and  $t_2$  divided by sum of  $\text{TA}_1$  and  $\text{TA}_2$  times 100 gives ml of 0.01 N acetic acid in aliquot sample in distillation flask before distillation, which, when multiplied by 3.20, gives  $\text{mg}$  of acetic acid in 100 g of sample being analyzed. If ratio  $t_2/t_1$  is less than CA by 0.2 (or more), an acid (or acids) of higher molecular weight than acetic is present. If identity of acids, other than formic, that may be present is established, the amounts can be determined by calculation, using simultaneous equations derived from ap-



propriate T values of distillation fractions (2), and distillation apparatus standardized with acids involved.

(2) *Formic acid absent*.—If ratio of titrations (corrected for blanks) of 200 ml to 50 ml distillates as determined under (b) agrees with CA ( $\pm 0.2$ ) calculate mg acetic acid in 100 g of sample being analyzed as follows: Divide sum of corrected titrations of 50 ml and 200 ml distillates by sum of  $TA_1$  and  $TA_2$  and multiply by 3.20.

If ratio is less than CA by 0.2 (or more) an acid (or acids) of higher molecular weight than acetic is present. If identity of acids, other than formic, that may be present is established the amounts can be determined by calculation, using simultaneous equations derived from appropriate T values of distillation fractions (2) and distillation apparatus standardized with acids involved.

#### SELECTED REFERENCES

- (1) J. Assoc. Official Agr. Chem., 25, 89 (1942).
- (2) Ibid., 21, 684 (1938); 25, 176 (1942).

## 25. FLAVORING EXTRACTS

### VANILLA EXTRACT AND ITS SUBSTITUTES

#### 25.1

##### SPECIFIC GRAVITY—OFFICIAL

Determine sp. gr. at 20/20° with a pycnometer as directed under 14.3.

#### 25.2

##### ALCOHOL—OFFICIAL

Proceed as directed under 16.6 or 16.7, or calculate from the sp. gr. of the distillate from the Wichmann Pb number, 25.9.

#### 25.3

##### GLYCEROL—TENTATIVE

Proceed as directed under 15.5, 15.6, or 15.7, selecting method according to quantity of sugar present. Use a quantity of the sample that contains 0.1–0.4 g of glycerol.

##### VANILLIN AND COUMARIN (GRAVIMETRIC) (1)—OFFICIAL

(Not applicable to concentrated vanillin and coumarin preparations in which quantity of vanillin and coumarin present in 50 ml exceeds quantity dissolved by 100 ml of H<sub>2</sub>O at 20°. With such preparations use smaller quantity of sample and dilute to 50 ml.)

#### 25.4

##### PREPARATION OF SOLUTION

Measure 50 ml of the extract at 20° into 250 ml beaker bearing marks showing volumes of 80 ml and 50 ml, dilute to 80 ml, and evaporate to 50 ml in water bath kept at 70° or below. Dilute again with H<sub>2</sub>O to 80 ml and evaporate to 50 ml. Transfer to 100 ml flask, rinsing beaker with hot H<sub>2</sub>O, add 25 ml of 8% neutral Pb acetate soln, make up to mark with H<sub>2</sub>O, shake, and allow to stand 18 hours (overnight) at 37–40°. Decant into small, dry filter, reserving filtrate (Soln A) for determination of vanillin and coumarin (25.5), Pb number (Winton, 25.8) and the residual color (25.19).

#### 25.5

##### DETERMINATION

(a) *Vanillin*.—Transfer a 50 ml aliquot of filtrate (Soln A) to separator and extract with 4 successive 15 ml portions of ether (previously washed twice with equal volume of H<sub>2</sub>O to remove alcohol). Wash combined ether solns 4 or 5 times with NH<sub>4</sub>OH (1+11), using 10 ml the first time and 5 ml thereafter. Reserve the ether soln for determination of coumarin. Slightly acidify combined ammoniacal solns with HCl (1+2), cool, and extract in a separator with 4 portions of washed ether, using ca 40 ml altogether. Evaporate ethereal solns at room temp., dry over H<sub>2</sub>SO<sub>4</sub>, and weigh. (Vanillin residue often appears first as oil-like droplets, which on standing crystallize into light colored masses.) If, after standing in desiccator, residue is considerably discolored or gummy, extract vanillin from it by treating with at least 15 successive portions of boiling petroleum benzine (b.p. 40° or below); combine the petroleum benzine extracts, evaporate to dryness, and weigh.

The residue, if pure vanillin, should be white crystals melting at ca 80°. Dissolve small quantity of residue in 2 drops of HCl and add a crystal of resorcin. Vanillin gives pink coloration.

(b) *Coumarin*.—Evaporate at room temp. the original ether extract obtained under (a), from which the vanillin has been removed by means of NH<sub>4</sub>OH (1+11), dry over H<sub>2</sub>SO<sub>4</sub>, and weigh.

The residue, if pure coumarin, melts at ca 67°. Dissolve a small quantity of the residue in not more than 0.5 ml of hot H<sub>2</sub>O and add a few drops of 0.1 *N* I. Coumarin yields a brown precipitate that finally gathers in green flecks, leaving a clear brown colored soln. The reaction is especially marked if reagent is applied with glass rod to few drops of the soln on a white plate or tile.

#### VANILLIN (COLORIMETRIC) (2)—OFFICIAL

##### 25.6

###### REAGENTS

(a) *Phosphotungstic-phosphomolybdic acid reagent*.—To 100 g of pure Na tungstate and 20 g of phosphomolybdic acid (free from nitrates and NH<sub>4</sub> salts), add 100 g of H<sub>3</sub>PO<sub>4</sub> and 700 ml of H<sub>2</sub>O. Boil over free flame 1½–2 hours, cool, filter if necessary, and make up with H<sub>2</sub>O to 1 liter. An equivalent amount of pure molybdic acid may be substituted for the phosphomolybdic acid.

(b) *Stock vanillin soln*.—Dissolve 1 g of vanillin in 250 ml of alcohol and dilute to 1 liter with H<sub>2</sub>O.

(c) *Standard vanillin soln*.—Dilute 10 ml of the stock vanillin soln to 100 ml with H<sub>2</sub>O. Use only freshly prepared solns.

##### 25.7

###### DETERMINATION

Transfer to 100 ml volumetric flask a quantity of sample that contains 8–12 mg of vanillin (usually 5 ml). Add 75 ml of tap H<sub>2</sub>O at room temp. and 4 ml of Pb soln (50 g each of basic and neutral Pb acetate/liter). Dilute to 100 ml with H<sub>2</sub>O and mix. Filter thru dry filter paper and pipet 5 ml of clear filtrate into 50 ml volumetric flask. Into another 50 ml volumetric flask pipet 5 ml of the standard vanillin soln. To each of these flasks add from a pipet 5 ml of the phosphotungstic-phosphomolybdic acid reagent, allowing it to flow down neck of flask in such a way as to wash down the vanillin that may be on sides of flask. Mix contents of flasks by rotating and after 5 min. dilute contents to 50 ml with saturated Na<sub>2</sub>CO<sub>3</sub> soln. Mix thoroly by inverting flasks several times and allow to stand at least 10 min. so that the precipitate that forms may separate completely. Filter solns thru dry filter papers and compare blue colors of clear solns in colorimeter. Report result as grams of vanillin/100 ml of extract.

#### LEAD NUMBER—OFFICIAL

##### 25.8

###### I. Winton Method (3)

Determine Pb as sulfate or chromate, 25.10(a) or (b), in filtrate from Pb acetate precipitate (Soln A, 25.4) and in filtrate from blank determination, using H<sub>2</sub>O and 5 drops of acetic acid in place of sample. Calculate Pb number and report as "Lead Number—Winton."

##### 25.9

###### II. Wichmann Method (4)

Place 175 ml of boiled H<sub>2</sub>O in a round-bottomed flask of 1 liter capacity. Add by means of pipet 25 ml of clear Pb acetate soln (8g/100 ml) and 50 ml of sample. Place flask in hole in an asbestos board that is large enough to prevent heating upper portion of flask. (When contents of flask are reduced to 50 ml of liquid, level of the liquid should be even with top of board, or slightly above it.) Connect flask to condenser, and with moderate flame distil 200 ml into volumetric flask, reserving distillate for determination of alcohol. Transfer residual soln to 100 ml volumetric flask by means of CO<sub>2</sub>-free H<sub>2</sub>O and a bent glass rod provided with a rubber tip. When cool, dilute to 100 ml with CO<sub>2</sub>-free H<sub>2</sub>O, mix, and filter thru dry filter (Soln A). Conduct blank determination, using 5 drops of acetic acid in place of sample and distilling

150 ml instead of 200 ml. Determine Pb as directed in 25.10(a) or (b); calculate Pb number and report as "Lead Number—Wichmann."

**25.10****DETERMINATION OF LEAD (4)**

(a) *As sulfate*.—Pipet 10 ml of Soln A (25.4 or 25.9) into 250 ml beaker and add 25 ml of  $H_2O$ , 2 ml of  $H_2SO_4$  (1+1), and 100 ml of alcohol; stir, and allow to settle overnight. Filter on Gooch crucible, wash with alcohol, ignite at low redness, cool in desiccator, and weigh. Difference between weight of  $PbSO_4$  obtained from blank and that obtained from sample  $\times 13.66$  = Pb number of the extract.

(b) *As chromate*.—Pipet 10 ml of Soln (25.4 or 25.9) into 400 ml beaker and add 2 ml of acetic acid, 25 ml of  $H_2O$ , and 25 ml of ca 0.1  $N$   $K_2Cr_2O_7$ . Heat beaker and contents immediately with moderate flame until precipitate changes in color from yellow to orange. Filter on Gooch crucible; wash thoroughly with hot  $H_2O$  and then with a few ml each of alcohol and ether. Dry at  $100^\circ$ , cool in desiccator, and weigh. Difference between weight of  $PbCrO_4$  obtained from blank and that obtained from sample  $\times 12.82$  = Pb number.

**25.11****TOTAL SOLIDS—OFFICIAL**

Proceed as directed under 34.4 or 34.5, using 10 ml of the sample.

**25.12****ASH—OFFICIAL**

Evaporate 10 ml of extract and proceed as directed under 34.9 or 34.10.

**25.13****ASH CONSTITUENTS.—See Chap. 12****25.14****SUCROSE—OFFICIAL.—See 34.23, 34.24, or 34.30****VANILLA RESINS****25.15****Quantitative Methods (5)—Tentative**

Pipet 50 ml of the extract into small beaker, add 50 ml of  $H_2O$ , and evaporate to 50 ml on steam bath. Add 50 ml of  $H_2O$  and again evaporate to 50 ml. Cool. If the mixture has an acid reaction, add 2 ml of  $HCl$  (1+1). If the mixture is not acid to litmus, add  $HCl$  (1+1), dropwise, until distinctly acid to litmus paper, then 1 ml in excess. Cover and let stand overnight. Filter, wash 6 or 7 times with ca 0.05  $N$   $HCl$  (9 ml of  $HCl$  (1+1) per liter of  $H_2O$ ). Dissolve resin in warm alcohol by pouring thru filter. Evaporate alcohol in tared 50 ml beaker and dry to constant weight at  $100^\circ$ . Report results to 2 decimal places only. Reserve resin for qualitative tests.

**25.16****Qualitative Tests—Tentative**

Place a portion of dried residue in a few ml of 5%  $KOH$  soln. Vanilla resins dissolve, giving a deep red soln. Acidify, and a precipitate is obtained.

Dissolve a portion of the dried residue in alcohol. To a portion of the soln add a few drops of 10%  $FeCl_3$  soln; to another portion add  $HCl$ . Neither produces any marked change in color if residue consists of vanilla resins. Most other resins in alcoholic soln give color reactions with  $FeCl_3$  or  $HCl$ .

To portion of filtrate obtained in 25.15, add a few drops of basic Pb acetate soln, 34.19(a). Owing to excessive quantity of organic acids, gums, and other extractive matter, the precipitate is so bulky as almost to solidify. Filtrate from this precipitate should be almost colorless.

Test another portion of filtrate from the resin for tannin with a soln of gelatin. Tannin is present in varying but small quantities, but should not be present in excessive quantities.

## 25.17

## METHYL ALCOHOL—OFFICIAL

Proceed as directed under 16.28, 16.29, or 16.34, using distillate from determination of alcohol, 25.2.

## 25.18

## COLOR VALUE—TENTATIVE

Pipet 2 ml of the extract into 50 ml volumetric flask and dilute to mark with a mixture of equal parts of alcohol and  $H_2O$ . Determine color value of this diluted extract in terms of red and yellow by means of Lovibond tintometer, using a 1" cell. To obtain color value of original extract multiply the figures for each color by 25.

## 25.19

RESIDUAL COLOR AFTER PRECIPITATION WITH LEAD  
ACETATE (6)—TENTATIVE

Determine color value, in terms of red and yellow, of filtrate from the Pb acetate precipitate obtained under 25.4, using 1" Lovibond cell. Multiply reading by 2 to reduce results to basis of original extract. If the actual reading of soln is greater than 5 red and 15 yellow, as may be the case if extract is highly colored with caramel, use  $\frac{1}{2}$  or  $\frac{1}{4}$  inch cell, and multiply readings, respectively, by 4 or 8. To obtain percentages of the two colors remaining in the Pb acetate filtrate, divide figures for red and yellow, respectively, by corresponding figures of original extract obtained under 25.18 and multiply quotients by 100. Calculate also ratio of red to yellow in both extract and Pb acetate filtrate.

## 25.20

## COLOR INSOLUBLE IN AMYL ALCOHOL—TENTATIVE

Proceed as directed under 16.37, using 25 ml of the extract and shaking with 25 ml of the Marsh reagent instead of 20 ml.

25.21 COLORING MATTERS OTHER THAN CARAMEL—TENTATIVE.—See Chap. 21.

## LEMON, ORANGE, AND LIME EXTRACTS AND FLAVORS

## 25.22

## SPECIFIC GRAVITY—OFFICIAL

Determine sp. gr. at 20/20° with a pycnometer, as directed under 16.4.

## ALCOHOL

## 25.23

## Method I.—Official

Pipet 50 ml of the extract into 200 ml volumetric flask, noting temp.; dilute with  $H_2O$  to ca 200 ml; and allow mixture to stand until oil separates in clear layer at top, or centrifuge and add  $H_2O$  to bring lower meniscus of oil to mark. Pour mixture into dry Erlenmeyer flask containing 5 g of light  $MgCO_3$ , stopper, shake well, and filter quickly thru large, dry, folded filter. Introduce 100 ml aliquot of filtrate, measured at same temp., into 300–500 ml distillation flask, and add 50 ml of  $H_2O$ . Attach flask to condenser and distil almost 100 ml. Add  $H_2O$  to complete volume of distillate to 100 ml at same temp., mix well, and determine sp. gr. at convenient temp. Ascertain corresponding percentage of alcohol by volume from 44.23 and multiply result thus obtained by 4 to obtain percentage of alcohol by volume in original sample.

## 25.24

## Method II (7)—Official

(Applicable to extracts consisting only of oil, alcohol, and water)

Let  $S$  represent sp. gr. of the extract at 20/20°, as determined under 25.22;  $O$ , sp. gr. of the oil; and  $p$ , percentage of oil found. Then  $100 - p$  = percentage of water-alcohol soln, the sp. gr. of which, represented by  $P$ , is calculated as follows:

$$S = \frac{Op + P(100 - p)}{100}, \quad \text{whence} \quad P = \frac{100S - Op}{100 - p}.$$

The value of  $E$ , the alcohol equivalent of  $P$ , is obtained from 44.23. It gives percentage of alcohol in the alcohol-water soln. To find percentage of alcohol in the extract, apply following formula:

$$\text{Percentage by volume of alcohol in extract} = E \left( 1 - \frac{p}{100} \right).$$

The value of  $O$  for lemon oil may be taken as 0.86 and for orange oil as 0.85.

**25.25****METHYL ALCOHOL—OFFICIAL**

Proceed as directed under 16.26, using distillate from determination of alcohol. 25.23.

**ISOPROPYL ALCOHOL—TENTATIVE**

(Applicable to lemon extract in the absence of acetone)

**25.26****PREPARATION OF SAMPLE**

Introduce a sample of extract that contains not over 8 g of total alcohols (an approximation to the alcoholic content may be had by a sp. gr. determination and reference to 44.23), into a separator containing in the stem a pledget of cotton wet with  $H_2O$ . Add 25 ml of 10%  $NaCl$  soln and 25 ml of petroleum benzine. Shake well and when layers have separated draw the lower layer into a flask. Repeat extraction with 3 more 25 ml portions of the  $NaCl$  soln or until the alcohol is completely extracted. Add  $H_2O$  to combined aqueous extracts until volume is ca 150 ml. Connect flask to vertical condenser and distil into 100 ml volumetric flask, removing flask when distillate is 2–3 ml below mark. Make to volume and mix.

**25.27****QUALITATIVE TEST FOR ACETONE**

To 2 ml of distillate add 5 ml of 5% alcoholic soln of *o*-nitrobenzaldehyde, and 1 ml of 10%  $NaOH$  soln. Mix, then shake with a small quantity of  $CHCl_3$ . A blue color in the  $CHCl_3$  shows presence of acetone.

**25.28****DETERMINATION**

Into a 500 ml Erlenmeyer flask containing 50 ml of ca 2  $N$   $K_2Cr_2O_7$  pipet 10 ml of distillate and add 100 ml of  $H_2SO_4$  (1 + 3). Stopper flask, swirl, and let stand 30 min. Add 100 ml of 25%  $FeSO_4$  soln. Connect flask to vertical condenser thru foam trap. Slowly distil ca 100 ml into 500 ml volumetric flask containing 200–300 ml of cold  $H_2O$ . Dilute to mark, mix, and pipet 25 ml into glass-stoppered flask containing 25 ml of normal  $NaOH$ ; add 50 ml of standard 0.1  $N$   $I$  while swirling flask. Allow to stand 15 min. Add 26 ml of normal  $HCl$  and at once titrate residual  $I$  with standard 0.1  $N$   $Na_2S_2O_3$ , adding starch soln when the  $I$  color is nearly discharged. Each ml of 0.1  $N$   $I$  consumed in the reaction = 0.001001 g of isopropyl alcohol ( $CH_3CHOHCH_3$ ).

**25.29****GLYCEROL—TENTATIVE**

Proceed as directed under 15.5, 15.6, or 15.7, selecting the method according to quantity of sugar present. Use a quantity of sample that contains 0.1–0.4 g of glycerol.

## OILS OF LEMON AND ORANGE IN EXTRACTS

## 25.30

*I. By Polarization—Official*

Without diluting, polarize the extract at 20° in 200 mm tube. Divide reading in degrees Ventske by 3.2 in the case of lemon extract and by 5.2 in the case of orange extract; in the absence of other optically active substances, the result will be the percentage of oil by volume. If cane sugar is present, determine as directed under 25.42 and correct reading accordingly. To obtain percentage of oil by weight from percentage by volume, multiply volume percentage by 0.86 in the case of lemon extracts, and by 0.85 in the case of orange extracts, and divide results by sp. gr. of original extract.

## 25.31

*II. By Precipitation—Official*

Pipet 20 ml of the extract into Babcock milk bottle. Add 1 ml of HCl (1+1), then 25–28 ml of H<sub>2</sub>O previously warmed to 60°. Mix, and let stand in H<sub>2</sub>O at 60° for 5 min. Centrifuge for 5 min., fill bottle with warm H<sub>2</sub>O to bring oil into graduated neck of flask, again centrifuge for 2 min., and place flask in H<sub>2</sub>O at 60° for a few minutes. Note percentage of oil by volume. If oil is present in amounts over 2%, add 0.4% to percentage of oil noted to correct for solubility of the oil. If less than 2% and more than 1% is present, add 0.3% for this correction. To obtain percentage of oil by weight from percentage by volume, multiply volume percentage by 0.86 in the case of lemon extracts, and by 0.85 in the case of orange extracts, and divide result by sp. gr. of original extract.

## 25.32

*III. By Precipitation in Presence of Mineral Oil—Tentative*

Proceed as directed under 25.75.

## OILS OF LEMON, ORANGE, OR LIME IN OIL BASE FLAVORS

*I. By Steam Distillation (8)—Official*

## 25.33

## APPARATUS

(a) *Steam generator filled with H<sub>2</sub>O.*—An oil can holding 1 gallon will serve the purpose.

(b) *Distillation flask.*—A Kjeldahl flask of ca 750 ml capacity, with shortened neck, ca 10" in height over all.

(c) *Spray tube.*—A glass tube with small perforated bulb at end passing thru rubber stopper and reaching to bottom of distillation flask.

(d) *Bent glass tube.*—About 8 mm in diam. Connects distillation flask to upright condenser. The shape of this tube allows vapor condensing in tube to return to distillation flask.

(e) *Liebig condenser.*—With 20" water jacket.

(f) *Wilson receiving flask.*—(Fig. 38). Shaped like Babcock test bottle with graduated neck but of much larger capacity and with vertical glass outlet tube sealed on near bottom. Upper end of outlet tube is turned down. Capacity of flask is ca 250 ml. The neck may consist of portion of buret graduated from 0–25 ml with top flared out. The outlet tube is ca 3 mm in diameter, and end is at such height that when flask is filled with H<sub>2</sub>O the meniscus in neck will be between 0 and 1 ml marks.

## 25.34

## DETERMINATION

Measure 100 ml of sample in graduated cylinder and transfer to distillation flask. Immerse flask in water bath and connect with condenser by means of the bent

glass tube. Fill receiving flask with  $H_2O$  and so place under condenser that end of condenser will be ca 0.5" above level of  $H_2O$  in receiving flask. Place 200 ml graduated cylinder under end of outlet tube to catch displaced liquid. Heat water bath to boiling and pass steam thru sample until 200 ml of liquid has been collected in graduated cylinder.

Disconnect apparatus, allow receiving flask to stand 15 min., or until separation of oil is complete, and read volume of oil obtained. Calculate percentage (by volume) of essential oil in sample by dividing reading by 0.90 for lemon oil in corn and cottonseed oils, 0.95 for orange oil in corn and cottonseed oils, and by 0.78 for distilled or expressed lime oil in corn and cottonseed oils. Where menstruum is mineral oil, subtract 0.3 ml from reading before dividing by factors 0.90, 0.95, and 0.78 for lemon oil, orange oil, and lime oil, respectively.

### 25.35 II. By Polarization (9)—Tentative

Polarize the sample at  $20^\circ$  in 200 mm tube, making 5 readings. From average of these readings in degrees Ventzke subtract, for corn oil  $+0.6^\circ$ , for cottonseed oil  $-0.3^\circ$ , for peanut oil  $+0.2^\circ$ , and for mineral oil  $+5.5^\circ$ , as correction for rotatory effect of menstruum. To obtain percentage by volume of essential oil in mixture, divide corrected polariscopic reading so obtained by factor 3.4 for lemon oil in corn oil, 3.7 for lemon oil in cottonseed oil, 3.6 for lemon oil in peanut oil, 3.5 for lemon oil in mineral oil, 5.4 for orange oil in corn oil, 5.7 for orange oil in cottonseed oil, 5.6 for orange oil in mineral oil, 2.0 for lime oil in corn oil, 2.3 for lime oil in cottonseed oil, and 2.2 for lime oil in mineral oil.

### TOTAL ALDEHYDES (10)—OFFICIAL

#### 25.36

#### REAGENTS

(a) *Aldehyde-free alcohol*.—Allow alcohol, containing 5 g of metaphenylenediamine hydrochloride/liter, to stand at least 24 hours with frequent shaking. (Nothing is gained by previous treatment with KOH.) Boil under reflux condenser at least 8 hours, longer if necessary; allow to stand overnight, and distil, rejecting first 10 and last 5 ml that come over. Store in dark, cool place in well-filled bottles; 25 ml of this alcohol, on standing 20 min. at  $14-16^\circ$  with 20 ml of the fuchsin-bisulfite soln, should develop only faint pink coloration. If a stronger color is developed, repeat treatment with metaphenylenediamine hydrochloride as above.

(b) *Fuchsin-bisulfite soln*.—Dissolve 0.5 g of fuchsin in 250 ml of  $H_2O$ , add an aqueous soln of  $SO_2$  containing 16 g of the gas, allow to stand until colorless or nearly so, and make up to 1 liter with  $H_2O$ . Let stand 12 hours before using and keep in refrigerator. This soln is liable to deteriorate and should be reasonably fresh when used.

(c) *Standard citral soln*.—Weigh 0.5 g of citral into 50 ml volumetric flask, make

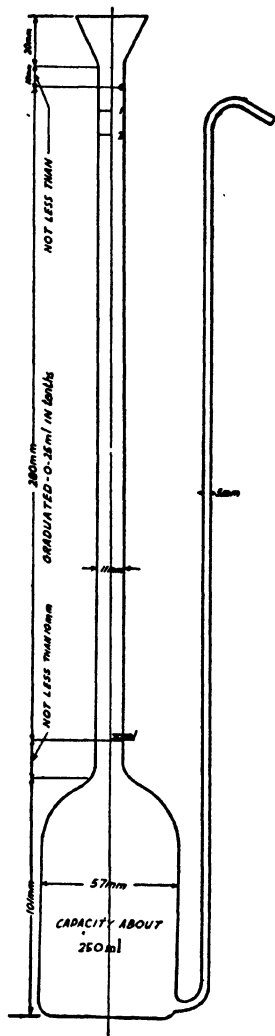


FIG. 38.—WILSON FLASK



up to mark with the aldehyde-free alcohol at room temp., stopper flask, and mix by shaking. Dilute 10 ml of this soln with the aldehyde-free alcohol to 100 ml in a volumetric flask, stopper flask, and mix by shaking. 1 ml of the dilute soln = 1 mg of citral.

## 25.37

## DETERMINATION

Weigh ca 25 g of the extract in stoppered weighing flask, transfer to 50 ml volumetric flask, and dilute to mark at room temp. with the aldehyde-free alcohol. Measure, at room temp., 2 ml (or other suitable quantity) of this soln into comparison tube. Add 25 ml of the aldehyde-free alcohol (previously cooled to 14–16°), then 20 ml of the fuchsin-bisulfite soln (also cooled), and finally make up to 50 ml mark with the aldehyde-free alcohol. Mix thoroly, stopper, and keep at 14–16° for 15 min. Prepare standard for comparison at same time and in same manner, using 2 ml of the standard citral soln, and compare colors developed. Calculate amount of citral present and repeat determination, using quantity sufficient to give sample approximately the strength of the standard. From this result calculate quantity of citral in sample. If comparisons are made in Nessler tubes, standards containing 1, 1.5, 2, 2.5, 3, 3.5, and 4 mg of citral may be prepared and trial comparison made against these, final comparison being made with standards lying between 1.5 and 2.5 mg with 0.25 mg increments.

It is absolutely essential to keep reagents and comparison tubes at required temp., 14–16°. If comparisons are made in a bath (possible only when bath is of glass), discard standards within 25 min. after adding the fuchsin-bisulfite soln. Give samples and standards identical treatment.

## CITRAL (11)—OFFICIAL

(Lemon and orange extracts)

## 25.38

## REAGENT

*Metaphenylenediamine hydrochloride-oxalic acid soln.*—Remove interfering colored impurities in metaphenylenediamine hydrochloride by digesting 3–5 g ca 5 min. with ca 25 ml of alcohol, decanting, and repeating 3 times. Dry crystals short time on steam bath. Dissolve 1 g in ca 45 ml of 85% alcohol, dissolve 1 g of crystallized oxalic acid in similar quantity of alcohol of same strength, and pour the two solns into 100 ml volumetric flask. Add 2 or 3 g of fullers' earth, dilute to mark with 85% alcohol, mix, and filter thru double folded filter.

## 25.39

## DETERMINATION

Weigh 25 g of the extract into 50 ml volumetric flask, dilute to mark with alcohol (95% by volume for extracts made with the oils; 50–95% by volume for terpeneless extracts) and mix. Pipet 2 ml or other suitable quantity of this soln into colorimeter tube, add 10 ml of the reagent, dilute to suitable volume, and compare resulting color with the colors of a set of standards containing known quantities of standard citral soln, 25.36 (c).

## 25.40

## TOTAL SOLIDS—OFFICIAL

Proceed as directed under 16.8, using 10 ml of the sample measured at 20°. -

## 25.41

## ASH—OFFICIAL

Ignite the residue from 10 ml of the extract as directed under 34.9 or 34.10.

25.42

## SUCROSE—OFFICIAL

Neutralize normal weight of the extract, evaporate to dryness, wash several times with ether, dissolve in  $H_2O$ , and proceed as directed under 34.23, 34.24, or 34.30.

25.43

## COLORING MATTERS—TENTATIVE.—See Chap. 21

25.44

## LEMON AND ORANGE PEEL COLOR—TENTATIVE

Place a few ml of the extract in each of 2 test tubes; to one, add slowly 3–4 volumes of  $HCl$  and to other, several drops of  $NH_4OH$ . If color is due to lemon or orange peel only, it is materially deepened by each treatment.

## LEMON AND ORANGE OILS

25.45

## SPECIFIC GRAVITY—OFFICIAL

Determine sp. gr. at  $20/20^\circ$  with a pycnometer as directed under 16.4.

25.46

## INDEX OF REFRACTION—OFFICIAL

Use any standard instrument, making the reading at  $20^\circ$ . See 31.8.

25.47

## OPTICAL ROTATION—OFFICIAL

Determine rotation at  $20^\circ$  with any standard instrument, 50 mm tube, and Na light. State results in angular degrees on 100 mm basis. If instruments having the sugar scale are used, the reading for orange oils is above the range of the scale, but readings may be obtained by the use of standard laevorotatory quartz plates, or by 25 mm tube. The true rotation cannot be obtained by diluting the oil with alcohol and correcting the rotation in proportion to the dilution.

## TOTAL ALDEHYDES (10)—OFFICIAL

25.48

*Fuchsin-Bisulfite Method*

Weigh a small quantity of the sample into small stoppered flask and dilute with aldehyde-free alcohol in proportion of 2 g of lemon oil or 4 g of orange oil to 10 ml of soln. Determine total aldehydes as directed under 25.37, expressing result as citral.

*Kleber Method (12)*

25.49

## REAGENT

*Phenylhydrazine soln.*—Prepare a 10% soln in absolute alcohol. Sufficiently pure phenylhydrazine can be obtained by distilling the commercial product in vacuo, rejecting the first portions coming over that contain  $NH_3$ .

25.50

## DETERMINATION

Weigh accurately ca 15 g of the sample into small, glass-stoppered flask, and add 10 ml of the phenylhydrazine soln. Allow to stand 30 min. at room temp. and titrate with 0.5 N  $HCl$ , using either methyl or ethyl orange indicator. Titrate similarly 10 ml of the phenylhydrazine soln. Difference in number of ml of 0.5 N acid used in these 2 titrations  $\times 0.076$  = weight of citral in sample. If difficulty is experienced in detecting end point of the reaction, titrate until soln is distinctly acid; transfer to separator and draw off alcoholic portion. Wash the oil with  $H_2O$ , adding washings to alcoholic soln, titrate back with 0.5 N alkali, and make necessary corrections.

## 25.51

*Hiltner Method (12)*

Weigh accurately ca 2 g of lemon oil or 8 g of orange oil into 100 ml volumetric flask, dilute to mark with alcohol, and proceed as directed under 25.39, using 2 ml of the dilute soln for the comparison.

## 25.52 PHYSICAL CONSTANTS OF THE 10 PER CENT DISTILLATE (15)—OFFICIAL

Place 50 ml of the sample in 3-bulb Ladenburg flask having main bulb 6 cm in diam. and of 120 ml capacity and the condensing bulbs of following dimensions: 3.5 cm, 3 cm, and 2.5 cm. Distance from bottom of flask to opening of side arm should be 20 cm. Distil the oil at rate of 2 ml/min. until 5 ml has been distilled. Determine refractive index and rotation of this distillate as directed under 25.46 and 25.47.

## 25.53

## PINENE (14)—OFFICIAL

*(Qualitative Test)*

Mix the 10% distillate, 25.52, with 5 ml of acetic acid, cool mixture thoroly in freezing bath, and add 10 ml of ethyl nitrite. Add slowly, with constant stirring, 2 ml of HCl (2+1). Keep mixture in freezing bath 15 min. Collect crystals formed on filter, using suction, and wash with alcohol. Return combined filtrate and washings to freezing bath for 15 min. Collect additional crystals formed on original filter. Wash combined crops of crystals thoroly with alcohol. Dry at room temp. and dissolve in minimum quantity of  $\text{CHCl}_3$ . Add methyl alcohol to the  $\text{CHCl}_3$  soln, a little at a time, until the nitroso-chlorides crystallize out. Mount the separated and dried crystals in olive oil and examine under microscope. Pinene nitroso-chloride crystals have irregular pyramidal ends, while limonene nitroso-chloride crystallizes in needles

## ALMOND EXTRACT

## 25.54

## ALCOHOL—TENTATIVE

As almond extract usually contains only ca 1 % of almond oil, in most cases the alcohol can be calculated from the sp. gr. of the extract. If the extract is high in solids, proceed as follows: Add 25 ml of the extract, measured at room temp., to 75 ml of saturated NaCl soln in separator, and extract twice with 50 ml portions of petroleum benzine (b.p. 40–60°). Collect the petroleum benzine extract in second separator and wash twice with 2 portions (25 ml) of saturated NaCl soln. Combine original NaCl soln with the washings, add a little powdered pumice, and distil into 100 ml volumetric flask. When almost 100 ml has been distilled, make up to mark with  $\text{H}_2\text{O}$  at room temp. and determine alcohol from the sp. gr. as directed under 16.6, using 44.23.

## 25.55

## BENZALDEHYDE—TENTATIVE

Measure out 2 portions of 10 ml each of the extract into 300 ml Erlenmeyer flasks and add 10 ml of phenylhydrazine soln (3 ml of acetic acid, 40 ml of  $\text{H}_2\text{O}$ , 2 ml of phenylhydrazine) to one flask and 15 ml to the other. Allow mixtures to stand overnight in dark place. Add 200 ml of  $\text{H}_2\text{O}$ , and filter thru weighed Gooch crucible provided with thin layer of asbestos. Wash precipitate first with cold  $\text{H}_2\text{O}$  and finally with 10 ml of 10% alcohol. Dry at 70° for 3 hours at pressure not to exceed 100 mm of Hg or to constant weight over  $\text{H}_2\text{SO}_4$ . Weight of precipitate  $\times 5.408$  = weight of benzaldehyde in 100 ml of sample. If duplicate determinations do not agree, repeat operation, using a larger quantity of the phenylhydrazine soln.

## 25.56

## BENZOIC ACID (15)—TENTATIVE

Measure 10 ml of the extract into 100 ml flask and add 10 ml of 10% NaOH soln and 20 ml of 3%  $\text{H}_2\text{O}_2$  soln; cover with watch-glass and place in water oven. Oxidation of the aldehyde to benzoic acid begins almost immediately and should be continued 5–10 min. after all odor of benzaldehyde has disappeared (20–30 min.). Remove flask from water oven; transfer contents to separator, rinsing off watch-glass; add 10 ml of  $\text{H}_2\text{SO}_4$  (1+5); and cool contents of funnel to room temp. under water tap. Extract the benzoic acid with 25, 25, 20, and 20 ml portions of ether, and wash combined extracts with 2 portions of 5–10 ml of  $\text{H}_2\text{O}$ , or until all  $\text{H}_2\text{SO}_4$  is removed. Filter into weighed dish, evaporate at room temp., dry overnight in desiccator, and weigh the benzoic acid. Multiply result by 10.

Multiply g/100 ml of benzaldehyde obtained under 25.55 by 1.151 to obtain equivalent of benzoic acid and subtract this product from g/100 ml of total benzoic acid obtained above. Difference = g of benzoic acid/100 ml of extract.

## HYDROCYANIC ACID

## 25.57

## Qualitative Test—Tentative

Add several drops of freshly prepared 3%  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  soln and a single drop of 1%  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  soln to several ml of the extract. Mix thoroly and add 10% NaOH soln, dropwise, until no further precipitate forms and then  $\text{H}_2\text{SO}_4$  (1+9) to dissolve the precipitate. In presence of even small quantities of HCN, a Prussian blue coloration or suspension will develop.

## 25.58 •

## Quantitative Method—Tentative

(In absence of chlorides)

Measure 25 ml of the extract into small flask and add 5 ml of freshly precipitated  $\text{Mg}(\text{OH})_2$ , Cl-free. Titrate with 0.1 N  $\text{AgNO}_3$ , using  $\text{K}_2\text{CrO}_4$  as indicator. 1 ml of 0.1 N  $\text{AgNO}_3$  = 0.0027 g of HCN.

## NITROBENZENE

## 25.59

## Qualitative Test—Tentative

Boil a few ml of the extract with some Zn dust and acetic acid and filter. Add to filtrate a drop of  $\text{CHCl}_3$ , make strongly alkaline with 10% NaOH soln, and heat. Presence of nitrobenzene in original extract is indicated by development of characteristic odor of phenylisocyanide.

## CASSIA, CINNAMON, AND CLOVE EXTRACTS

## 25.60

## ALCOHOL—TENTATIVE.—See 25.54

## 25.61

## ISOPROPYL ALCOHOL—TENTATIVE

Proceed as directed under 25.26–25.28.

## OIL (15)—TENTATIVE

## 25.62

## Method I

Transfer 10 ml of the extract to separator, add 30 ml of  $\text{H}_2\text{O}$ , acidify with 1 ml of HCl (1+1), and extract 3 times with ether, using not less than 100 ml altogether. Wash combined ether solns twice with  $\text{H}_2\text{O}$ , and in the case of cinnamon extract dry by shaking with small quantity of granulated  $\text{CaCl}_2$ . Transfer to weighed

wide-mouthed weighing bottle and evaporate the ether as rapidly as possible on boiling water bath, rotating liquid onto sides of bottle to rid residual oil of traces of ether. Weigh residue and divide weight by sp. gr. of the oil in order to obtain percentage of oil by volume. In the case of clove oil, allow weighing bottle to remain in balance case until usual film of moisture has evaporated. The time of weighing, however, should not be delayed over 3 min. Determine refractive index of residual oils at 20°. Dissolve a drop of the oil in several drops of alcohol and add a drop of 10%  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  soln.

*Specific gravity, refractive index at 20°, and color reaction with  $\text{FeCl}_3$  soln*

OIL	SPECIFIC GRAVITY	REFRACTIVE INDEX AT 20°	COLOR REACTION WITH $\text{FeCl}_3$ SOLN
Cassia . . . . .	1.05	1.585–1.600	Brown
Cinnamon . . . . .	1.03	1.590–1.599	Green
Cloves	1.055	1.560–1.565	Deep blue

## 25.63

*Method II (16)*

Pipet 10 ml of the extract into standard Babcock milk bottle. Remove nearly all alcohol by blowing air into bottle thru small glass tube 30 min., or longer if necessary. Add from 10 ml buret 1 ml of solvent (equal parts of U.S.P. mineral oil and  $\text{H}_2\text{O}$ -free kerosene), shake well, and fill with saturated  $\text{MgSO}_4$  soln. Centrifuge 10 min. and read volume of oil from extreme bottom to extreme top of column. To obtain percentage of oil subtract 5 divisions and multiply remainder by 2.

## GINGER EXTRACT

## 25.64

ALCOHOL—TENTATIVE.—See 16.6 or 16.7

## 25.65

SOLIDS—TENTATIVE

Evaporate 10 ml of the extract nearly to dryness on steam bath, dry 2 hours in water oven at temp. of boiling  $\text{H}_2\text{O}$  and weigh.

## 25.66

GINGER (QUALITATIVE TEST)—TENTATIVE

Dilute 10 ml of the extract to 30 ml, evaporate to 20 ml, decant into separator and extract with equal volume of ether. Allow ether to evaporate spontaneously in porcelain dish, and to residue add 5 ml of 75%  $\text{H}_2\text{SO}_4$  and ca 5 mg of vanillin. Allow to stand 15 min. and add an equal volume of  $\text{H}_2\text{O}$ . In the presence of ginger extract an azure blue color develops.

## 25.67

CAPSICUM (QUALITATIVE TEST)—TENTATIVE

To 10 ml of the extract add cautiously  $\text{NaOH}$  soln (1+9) until soln reacts very slightly alkaline with litmus paper. Evaporate at ca 70° to ca  $\frac{1}{2}$  original volume and render slightly acid with  $\text{H}_2\text{SO}_4$  (1+9), testing with litmus paper. Transfer to separator, rinsing dish with  $\text{H}_2\text{O}$ , and extract with equal volume of ether, avoiding formation of emulsion by shaking separator gently 1–2 min. Draw off lower layer and wash ether extract once with ca 10 ml of  $\text{H}_2\text{O}$ . Transfer washed ether extract to small evaporating dish, render decidedly alkaline with 0.5 *N* alcoholic  $\text{KOH}$ , and evaporate at ca 70° until residue is pasty. Add ca 20 ml more of the 0.5 *N* alcoholic  $\text{KOH}$  and allow mixture to stand on steam bath until gingerol is completely saponified (ca 30 min.). Dissolve residue in a little  $\text{H}_2\text{O}$  and transfer with  $\text{H}_2\text{O}$  to small separator. The volume should not exceed 50 ml. Extract alkaline

soln with equal volume of ether. Wash ether extract repeatedly with small quantities of  $H_2O$  until no longer alkaline to litmus. Transfer washed extract to small evaporating dish and allow ether to evaporate spontaneously. Finally test residue for capsicum by moistening tip of the finger, rubbing it on bottom and sides of dish, and then applying finger to end of tongue. A hot, stinging, or prickly sensation, which persists for several minutes, indicates capsicum or other foreign pungent substances.

### PEPPERMINT, SPEARMINT, AND WINTERGREEN EXTRACTS

25.68

ALCOHOL—TENTATIVE.—See 25.54

25.69

ISOPROPYL ALCOHOL—TENTATIVE.—See 25.26–25.28

#### OIL (17)—TENTATIVE

25.70

##### *Method I*

Pipet 10 ml of the extract into Babcock milk bottle, add 1 ml of  $CS_2$ , mix thoroly, and add 25 ml of cold  $H_2O$  and 1 ml of  $HCl$ . Close mouth of bottle and shake vigorously; centrifuge 6 min., and remove all but 3–4 ml of supernatant liquid, which should be practically clear, by aspirating thru glass tube of small bore. Connect stem of bottle with filter pump and immerse bottle in  $H_2O$  kept at ca  $70^\circ$  for 3 min., removing from bath every 15 seconds and shaking vigorously. Continue in same manner for 45 seconds, using boiling water bath. Remove from bath and shake while cooling. Disconnect from the suction and fill bottle to neck with saturated  $NaCl$  soln at room temp., centrifuge for 2 min., and read volume of separated oil from top of meniscus. Multiply reading by 2 to obtain percentage of oil by volume. In the case of wintergreen, use as floating medium a mixture of 1 volume of  $H_2SO_4$  and 3 of saturated  $Na_2SO_4$  soln.

25.71

*Method II.*—See 25.75

25.72

METHYL SALICYLATE IN WINTERGREEN EXTRACT (18)—TENTATIVE

Mix 10 ml of the extract with 10 ml of 10%  $KOH$  soln. Heat on steam bath until volume is reduced ca one-half. Add distinct excess of  $HCl$  (1+1), cool, and extract with 40, 30, and 20 ml portions of ether. Filter extract thru dry filter into weighed dish, wash paper with 10 ml of ether, and allow filtrate and washings to evaporate spontaneously. Dry in desiccator containing  $H_2SO_4$  and weigh. Weight of salicylic acid so found  $\times 9.33$  = percentage by volume of methyl salicylate in sample.

### ANISE AND NUTMEG EXTRACTS

#### OIL (16)—TENTATIVE

25.73

##### *Method I*

To 10 ml of the extract in Babcock milk bottle, add 1 ml of  $HCl$  (1+1), then sufficient half-saturated  $NaCl$  soln, previously heated to  $60^\circ$ , to fill flask nearly to neck. Cork and let stand in  $H_2O$  at  $60^\circ$  ca 15 min., rotate occasionally, and centrifuge 10 min. at ca 800 r.p.m. Fill bottle to neck with saturated  $NaCl$  soln and again centrifuge 10 min. If separation is not satisfactory or liquid is not clear, cool to ca  $10^\circ$  and centrifuge for an additional 10 min. Reading  $\times 2$  = percentage of oil by volume.

25.74

*Method II.*—See 25.75

## OTHER EXTRACTS AND TOILET PREPARATIONS

## 25.75

## ESSENTIAL OIL (18)—TENTATIVE

(Applicable to extracts of allspice, anise, caraway, lemon, nutmeg, orange, peppermint, pimiento, rosemary, thyme, wintergreen, and methyl salicylate.)

Pipet 10 ml of sample (5 ml when oil content exceeds 5% by volume) into standard Babcock milk bottle, add 0.50 ml of solvent (equal parts of U.S.P. mineral oil and H<sub>2</sub>O-free kerosene) and 1 ml of IICI (1+1), and fill to shoulder with saturated NaCl soln. Shake bottle 3 min., then add the NaCl soln to bring column of oil within graduations on neck. Centrifuge 10 min. at high speed and read volume of oil from extreme bottom to extreme top of column. (Read from extreme bottom to bottom of meniscus at top of column for allspice, peppermint, and pimiento extracts.) To obtain percentage of oil subtract 2.5 divisions and multiply remainder by 2. (Multiply by 4 if 5 ml sample is used.)

 $\beta$ -IONONE (19)—OFFICIAL

## 25.76

## Method I

(Applicable to pure solns containing 100 mg or less in 5 ml of alcohol)

Place 5 ml of alcohol containing 10–100 mg of  $\beta$ -ionone in 125 ml conical flask. Add 95–100 mg of solid *m*-nitrobenzhydrazide and dissolve by warming soln on steam bath, taking precautions to prevent loss of alcohol thru evaporation. Add 5 ml of H<sub>2</sub>O, and if soln becomes cloudy, warm until clear. Remove soln from steam bath, add 0.2 ml of acetic acid, stopper flask lightly, and place upon a wooden surface to prevent too rapid cooling. If ca 20 mg or more of  $\beta$ -ionone is present, crystals will begin to form within 30 min. after contents of flask have reached room temp. Let stand in room at least 2 hours (overnight does no harm) and add 5 ml of H<sub>2</sub>O dropwise, mixing soln continuously during the addition by rotating flask. Stopper, let stand in room at least 1 hour, and place in refrigerator overnight (not longer than 48 hours). Filter thru No. 3 or 4 sintered glass crucible, wash with 30 ml of dilute alcohol (3+7), using a wet policeman to remove precipitate adhering to flask, and dry at 100°. Weight of precipitate  $\times 0.541$  = weight of  $\beta$ -ionone. Identify crystals microscopically, 25.79.

## Method II

(Applicable to raspberry concentrates)

## 25.77

## APPARATUS

(a) *Steam generator filled with water.*—Oil can holding 1 gallon will serve purpose.

(b) *Distillation flask.*—Round-bottomed boiling flask having interchangeable ground-glass connection 24/40, capacity about twice the volume of sample to be used.

(c) *Still head.*—Adapter, 75° angle, with interchangeable male connections 24/40 at bottom and side and female connection 14/35 at top, with side arm lengthened and bent to fit vertical condenser.

(d) *Spray tube.*—Adapter, for use with Woulff bottles equipped with interchangeable ground-glass connection, aeration tube with connection 14/35, holes in bulb ca 2 mm in diam., length of tubing such that when apparatus is set up, the bulb is situated not more than 20 mm above bottom of distilling flask.

(e) *Condenser.*—Coil type with interchangeable female connection 24/40 at top with 250 to 300 mm jacket and outlet tube lengthened to ca 200 mm to reach bottom of receiving flask.

(f) *Receiving flask.*—Conical flask of 500 ml capacity.

## 25.78

## DETERMINATION

Place 250–1000 ml of sample (should contain not more than 100 mg of  $\beta$ -ionone) in distilling flask and connect with apparatus. Add enough  $H_2O$  to receiving flask to just cover outlet of condenser. Heat sample nearly to boiling on asbestos mat with flame or by immersing it in boiling water bath. As soon as sample has reached temp. of bath or has just begun to boil, connect with steam generator and pass rapid current of steam thru sample until ca 500 ml of distillate has been collected.

Add sufficient  $H_2O$  to distillate to reduce alcohol content to ca 10% or less and transfer to large separator. Add 150–200 ml of ether, depending upon volume of soln, so that ca 100 ml will be obtained upon separation.

Shake thoroly ca 2 min. After allowing mixture to settle till clear, draw off watery soln till ca 25 ml remains in separator. Whirl liquid and again allow to settle. When clear, draw off remainder of water layer, then draw off ether soln into 125 ml conical flask containing 95–100 mg of *m*-nitrobenzhydrazide. After separator has drained ca 1 min., close stopcock, pour 10–15 ml of ether into separator to wash down sides, and allow soln to settle 1 min., then add to main ether soln. Add 0.2 ml of acetic acid and dissolve the solid reagent by stirring and breaking up lumps with glass rod, warming if necessary to complete soln. Permit mixture to stand ca 1 hour and evaporate on steam bath to ca 10 ml, passing current of air into flask to hasten evaporation and keep down temp. In meantime make second extraction of distillate, using 100 ml of ether. Add the separated ether soln to flask containing residue from first ether extract, follow with ether washings of separator, and after allowing to stand at least 15 min. evaporate to 10 ml as before. Similarly make third extraction, using 100 ml of ether, add to flask and evaporate as before until 1–3 ml of watery liquid and perhaps some oily residue remain.

While flask is still warm, add 5 ml of alcohol from pipet, allowing liquid to wash down sides of flask, and dissolve residue completely by warming on steam bath, protecting liquid against loss by evaporation. Add 5 ml of  $H_2O$  and warm if necessary to obtain a clear soln. Add 0.2 ml of acetic acid, close with cork stopper, and place flask on a wooden surface to prevent too rapid cooling.

After 2 hours add 5 ml of  $H_2O$  dropwise, mixing liquid by continuously rotating flask, stopper, and keep at room temp. at least 1 hour (overnight does no harm), then place in refrigerator overnight (not longer than 48 hours).

Filter on fritted glass crucible of porosity 3 or 4 and wash with ca 30 ml of dilute alcohol (3+7). Dry in vacuum oven at 70° and weigh. Weight of precipitate  $\times 0.541$  = weight of  $\beta$ -ionone. Identify crystals microscopically, 25.79.

If precipitated material consists of oily matter mixed with crystalline matter, place fritted glass crucible in Gooch holder attached to suction flask. By means of wire, support a test tube within the suction flask in such manner as to catch any liquid that may pass thru crucible. Add ca 5 ml of petroleum benzene, cover crucible, and let stand ca 5 min. Turn on suction just long enough to carry thru any solvent that may remain in crucible. Transfer petroleum benzene soln to small beaker and allow to evaporate spontaneously. Repeat several times until no more soluble matter is obtained by the extraction. Examine remaining contents of crucible and the several residues microscopically for crystals of  $\beta$ -ionone-*m*-nitrobenzhydrazide.

## 25.79

OPTICAL PROPERTIES OF  $\beta$ -IONONE-*m*-NITROBENZHYDRAZIDE (#1)

To the naked eye this substance in mass has a yellowish color, but when examined in ordinary light under microscope, it is essentially colorless and crystallizes in thin, rod-like plates, many having lath-like or frayed ends, some having six-sided outline. In parallel polarized light (crossed nicols), the extinction is parallel and the sign of elongation negative. The refractive indices are the minimum and maximum



values,  $n_{\alpha}=1.548$ , invariably shown on the elongated fragments when their long dimension is parallel to vibration plane of lower nicol (lengthwise), and  $n_{\gamma}=1.648$ , usually shown on elongated fragments when their long dimension is at right angles to vibration plane of lower nicol (crosswise).

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## 26. FRUITS AND FRUIT PRODUCTS

### 26.1

#### SAMPLING (1)—TENTATIVE

*Boxed dried fruit.*—Remove cover, bottom, or one side of box, as is most convenient. Remove a block comprising  $\frac{1}{4}$  of contents of box taken from one corner as follows: With sharp knife make vertical cut midway between *ends* of box to center of top surface, this cut to extend half way to the bottom. Make another vertical cut midway between *sides* of box, extending half way to the bottom, and continue it until it meets the first cut. Remove all fruit included in the angle formed by the two cuts. Working rapidly, break up lumps, thoroly mix, and take sufficient sample to fill quart Mason jar, replacing remainder in box. Seal jar and send to laboratory. Sample sufficient number of boxes taken from different parts of pile to constitute at least the square root of the lot.

### 26.2

#### PREPARATION OF SAMPLE—OFFICIAL

Without delay transfer samples received in open packages (*i.e.*, not in sterile condition) to glass-stoppered containers and keep in cool place. Make the determinations of alcohol, total and volatile acids, solids, and sugars, particularly in the case of fruit juices and fresh fruits, at once, as fermentation is liable to begin very soon. (Portions for determination of sucrose and reducing sugars may be weighed and kept for several days without fermenting if the slight excess of neutral Pb acetate soln required in the determination is added.) Prepare the various products for analysis as follows:

(a) *Juices.*—Mix thoroly by shaking to insure uniformity in sampling and filter thru muslin previously washed and dried. Prepare fresh juices by pressing the well-pulped fruit in jelly bag and filtering thru muslin previously washed and dried. Express juice of citrus fruit by one of common devices used for squeezing oranges or lemons, and strain expressed juice thru muslin previously washed and dried.

(b) *Jellies and sirups.*—Mix thoroly to insure uniformity in sampling.

(b<sub>1</sub>) *Preparation of soln.*—Weigh 300 g of the thoroly mixed sample into 2 liter flask and dissolve in H<sub>2</sub>O, heating on steam bath if necessary. Apply as little heat as possible to minimize inversion of sucrose. Cool, dilute to mark, mix thoroly by shaking, and use aliquots for the various determinations. If insoluble material is present, mix thoroly and filter before taking aliquots.

(c) *Fresh fruits, dried fruits, preserves, jams, and marmalades.*—Pulp by passing thru food chopper, or by use of Waring Blendor, Hobart Mixer, or other suitable mechanical mixing apparatus, or by grinding in large mortar, and mixing thoroly, completing operation as quickly as possible to avoid loss of moisture. In the case of dried fruits, pass sample thru food chopper three times, mixing thoroly after each grinding. Set the burrs or blades of food chopper as close as possible without crushing seeds. If container is No. 10 can or smaller, grind entire contents. Mix contents of larger containers thoroly by stirring and remove portion for grinding. In the case of stone fruits, remove pits, and determine their proportion in weighed sample.

(c<sub>1</sub>) *Preparation of soln.*—Weigh into 1.5–2 liter beaker 300 g of sample, well-pulped by means of Waring Blendor or other suitable type of mechanical grinder and mixed; add ca 800 ml of H<sub>2</sub>O; and boil 1 hour, replacing at intervals the H<sub>2</sub>O lost by evaporation. Transfer to 2 liter volumetric flask, cool, dilute to volume, and filter. With unsweetened fruit it is desirable, tho not actually necessary, to add sugar before

boiling; therefore weigh 150 g of fruit, add 150 g of sugar and 800 ml of  $H_2O$ , and proceed as directed previously.

(d) *Canned fruits*.—See 35.1. Carefully invert by hand all fruits having cups or cavities if they fall on sieve with cups or cavities up. Cups or cavities in soft products may be drained by tilting the sieve, but no other handling of these products while draining is permissible. Examination of the sirup in which the fruits are preserved is often sufficient. Separate liquor by draining, 35.1, and treat as directed under (a).

**26.3****ALCOHOL—OFFICIAL**

Determine alcohol in 50 g of the original material as directed under 15.4.

**26.4****MOISTURE (2)—OFFICIAL**

*Dried fruits*.—Spread 5–10 g of the prepared sample, 26.2 (c), as evenly as possible over bottom of metal dish ca 8.5 cm in diam. and provided with a tightly fitted cover, weigh, and dry at 70° for 6 hours under pressure not to exceed 100 mm of Hg. During drying admit to oven slow current of air (ca 2 bubbles/second) dried by passing thru  $H_2SO_4$ . (The metal dish must be placed in direct contact with metal shelf of oven.) Replace cover, cool dish in desiccator, and weigh. Disregard any temporary drop of oven temp. that may occur during early part of drying period owing to rapid evaporation of  $H_2O$ . With raisins and fruit similarly rich in sugar, use ca 5 g of sample and dry and weigh with dish ca 2 g of finely divided asbestos. Moisten with hot  $H_2O$ , mix sample and asbestos thoroly, evaporate on steam bath barely to dryness, and complete drying as directed above.

**TOTAL SOLIDS—OFFICIAL****26.5****I. Insoluble Matter Present**

*Fresh and canned fruits, jams, marmalades and preserves*.—Weigh accurately into large flat-bottomed dish 20 g of pulped fresh fruit, or a quantity of fruit products that will give not more than 3–4 g of dry material. If necessary to secure a thin layer of the material, add a few ml of  $H_2O$  and mix thoroly. Dry at 70° under pressure not to exceed 100 mm of Hg until consecutive weighings made at intervals of 2 hours do not vary more than 3 mg.

**26.6****II. No Insoluble Matter Present**

*Fruit juices, jellies and sirups*.—Proceed as directed under 34.4, 34.6, 34.7, or 34.8, using sample prepared as directed under 26.2(a) or (b).

**26.7****WATER-INSOLUBLE SOLIDS (3)—TENTATIVE**

For use with Büchner funnel, prepare filtering medium consisting of either a circular disk of absorbent cotton ca 80 mm diam., weighing ca 1.5 g, or of a coarse, qualitative filter paper 7 cm diam. For use with 60° funnel, prepare absorbent cotton circle ca 12.5 cm in diam. weighing ca 2 g or a 12½ cm filter paper. Wash filtering medium selected with hot  $H_2O$ , and dry overnight at 100–110° in open, flat-bottomed Al dish, 70 mm in diam., 30 mm deep, provided with close-fitting cover. Cool closed dish and contents 1 hour in desiccator and weigh to nearest mg.

Weigh 25 or 50 g of well-mixed sample, 26.2(c), to nearest centigram, transfer to 400 ml beaker, make to ca 200 ml mark with hot  $H_2O$ , mix, and boil gently 30 min., replacing at intervals  $H_2O$  lost by evaporation. Filter by gravity thru prepared cotton or filter paper, and keep water-insoluble solids from forming closely adhering

mat on surface of filtering medium by frequent additions of portions of sample. Wash with ca 800 ml of hot  $H_2O$ , loosening water-insoluble solids from filter with each addition. Remove excess  $H_2O$  from cotton by gently squeezing it on 60° funnel, or by application of suction on the Büchner funnel. Transfer to original weighing dish, and wipe off any remaining portions of water-insoluble solids on filter or funnel with previously weighed portion of prepared filtering medium. Dry overnight at 100–110°, cool for 1 hour in desiccator, and weigh.

**26.8 SOLUBLE SOLIDS IN FRESH AND CANNED FRUITS, JAMS, MARMALADES AND PRESERVES (4)—TENTATIVE**  
(Insoluble matter present)

Proceed as directed under 34.8. Percentage of soluble solids = % of solids determined by refractometer  $\times \frac{100 - b}{100}$ , in which  $b$  = water-insoluble solids.

**26.9 ASH—OFFICIAL**

Proceed as directed under 34.9 or 34.10, the temp. of ashing not to exceed 525°, using 25 g of juices, fresh fruits, or canned fruits, and 10 g of jellies, sirups, preserves, jams, marmalades, or dried fruits.

If the ash of the water-soluble portion only is desired, evaporate on steam bath to dryness 100 ml of the prepared soln, 26.2(b<sub>1</sub>) or (c<sub>1</sub>), and proceed as directed under 34.9 or 34.10.

**26.10 ALKALINITY OF THE ASH—OFFICIAL**

Into the Pt dish containing the ash obtained under 26.9 introduce a measured excess of 0.1 *N* HCl, warm on steam bath, cool, add a few drops of methyl orange indicator, and titrate excess acid with 0.1 *N* NaOH. Report as alkalinity, number of ml of 0.1 *N* acid required to neutralize the ash from 100 g of sample, and as alkalinity number, number of ml of *N* acid required to neutralize 1 g of ash. Reserve soln. for determination of S in ash.

**26.11 SULFUR IN ASH—OFFICIAL**

(For products containing a basic ash)

Add 5 ml of HCl (1 + 2.5) to the soln remaining after determination of alkalinity of ash, 26.10, and evaporate to dryness. Heat to 110° for 1 hour to dehydrate any  $SiO_2$ . Take up in 5 ml of the dilute HCl and filter, washing filter paper well with hot  $H_2O$ . Heat filtrate to boiling and add dropwise from buret or pipet 5 ml of 10%  $BaCl_2$  soln. Evaporate to 100 ml and let stand overnight. Filter on weighed Gooch or Munroe crucible or on 7 cm ashless filter paper, wash with hot  $H_2O$  until filtrate is free from chlorides, dry, ignite over Bunsen burner, and weigh as  $BaSO_4$ . As quantity of precipitate is small, exercise great care and make determination in duplicate. Report result as mg of S/100 g.

**26.12 TOTAL SULFUR (5)—TENTATIVE**

(For sulfured products and for samples containing little ash or an acidic ash)

In casserole as large as can be placed in electric muffle furnace available, place 1–3 g of  $MgO$  (1 g for fruit juices, 3 g for heavily sugared products and for dried fruits) or an equivalent quantity of  $Mg(NO_3)_2 \cdot 6H_2O$ , 1 g of powdered sucrose, and 50 ml of  $HNO_3$ . Add 5–10 g of the prepared sample, 26.2(a), (b), (c), or (d). Place the

same quantities of the reagents in another casserole for blank. Evaporate on steam bath to pasty consistency. Place casserole in cold electric muffle and gradually heat (not above 525°) until all  $\text{NO}_2$  fumes have been driven off. (All organic matter will have been destroyed.) Cool, dissolve and neutralize with  $\text{HCl}$  (1+2.5), adding an excess of ca 5 ml. Filter, heat to boiling, and add dropwise 5 ml of 10%  $\text{BaCl}_2$  soln. Evaporate to 100 ml, allow to stand overnight, filter, wash, ignite, and weigh the  $\text{BaSO}_4$ . Correct result for the  $\text{BaSO}_4$  obtained in the blank and report as mg of S/100 g. (The determination should be made in a room free from S fumes.)

## 26.13 CHLORINE IN ASH (6)—TENTATIVE.—See 12.41–12.44

### POTASSIUM (7)—OFFICIAL

## 26.14 ASHING OF SAMPLE

(a) *Slow Ashing*.—Ash 15–30 g of sample (representing ca 15 g of fruit) as directed under 26.9.

(b) *Rapid Ashing*.—To 15–30 g of sample in Ni or Pt dish (preferably flat-bottomed,  $3\frac{1}{2}$ " in diam., and 1" high), add 1 ml of 25%  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  soln; evaporate, char, and heat at temp. not exceeding 550° until C is removed (ca 15 min.). Cover dish with watch-glass and cool.

### Chloroplatinate Methods

## 26.15

### REAGENTS

(a) *Chloroplatinic acid soln*.—Dissolve 4.4 g of  $\text{H}_2\text{PtCl}_6$  ( $\approx 2.1$  g of Pt) in  $\text{H}_2\text{O}$  and dilute to 100 ml. 1 ml of this soln = 10 mg of  $\text{K}_2\text{O}$ . Use ca 20% excess.

(b) *Calcium carbonate suspension*.—Mix 50 ml of alcohol with 50 ml of glycerol and add 50 g of  $\text{CaCO}_3$  powder. Keep in dropping bottle and shake vigorously before using.

(c) *Calcium formate suspension*.—Mix 50 ml of alcohol with 50 ml of glycerol and add 50 g of  $\text{Ca}(\text{CHO}_2)_2$  fine crystals. Keep in dropping bottle and shake vigorously before using.

(d) *Alcoholic sodium hydroxide and sodium formate soln*.—Shake  $\text{NaOH}$  pellets with alcohol containing 0.2 ml of formic acid/100 ml until saturated.

## 26.16

### PREPARATION OF ASH SOLUTION

Wet down the ash, 26.14(a) or (b), with 5–10 ml of  $\text{H}_2\text{O}$ , cover dish with watch-glass, and acidify with slight excess of  $\text{HCl}$  (1+4) (2–3 ml for 26.14(a) and 4–5 ml for 26.14(b)).

## 26.17

### DETERMINATION

(a) *Gravimetric chloroplatinate method*.—Rinse watch-glass into dish and evaporate ash soln to dryness on steam bath. Add 5 drops of  $\text{HCl}$  (1+1) to residue. Add 5–10 ml of hot  $\text{H}_2\text{O}$  and rub sides and bottom of container with policeman. Transfer ash soln to 250 ml beaker with 50–75 ml of hot  $\text{H}_2\text{O}$ , add a few glass beads, and heat to boiling. Make distinctly alkaline with  $\text{NH}_4\text{OH}$  and add sufficient saturated  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  soln for complete precipitation (usually not more than 1 ml), cover beaker, and heat until precipitate becomes sufficiently granular to filter readily (incipient boiling for 30 min. is usually sufficient). Filter thru 5 or 7 cm fine-textured filter paper into large Pt dish and wash thoroly with hot  $\text{H}_2\text{O}$  (5–6 fillings of filter are usually sufficient).

Evaporate soln nearly to dryness on steam bath and add 1 ml of  $\text{H}_2\text{SO}_4$  (1+1). So rotate dish that the  $\text{H}_2\text{SO}_4$  comes in contact with all residue, adding a little  $\text{H}_2\text{O}$  if necessary. Return dish to steam bath and evaporate all the  $\text{H}_2\text{O}$  possible at that temp. Then heat dish, preferably on hot plate, at temp. of ca  $150^\circ$  until bubbling caused by decomposition of oxalates ceases, and gradually increase temp. until the  $\text{H}_2\text{SO}_4$  has evaporated. (When properly controlled, this treatment usually takes 45–90 min.) Heat sample cautiously over burner, being careful to avoid loss due to sputtering during decomposition of  $\text{NH}_3$  compounds. Finally heat dish to redness to remove traces of  $\text{NH}_3$  compounds and complete the ignition. Add 5 drops of  $\text{HCl}$  (1+1) to the residue.

Transfer the ash soln to round-bottomed porcelain dish of 100–200 ml capacity, using ca 50 ml of hot  $\text{H}_2\text{O}$ . Add a small excess of the  $\text{H}_2\text{PtCl}_6$  soln. Place mixture on steam bath and rotate dish from time to time to prevent precipitate from baking on side of dish, and evaporate to pasty consistency. (It is advisable to start the evaporation with several of the steam bath rings removed, and as concentration progresses to replace the rings so that heat is applied only to that surface of dish that is covered by liquid.) Avoid exposure to  $\text{NH}_3$  fumes at all times. Add ca 50 ml of 90% alcohol to dish and transfer to Gooch crucible containing suitable asbestos mat, or 30 ml Gooch crucible with medium porosity fritted disk. Wash 8 or 10 times with 20 ml portions of 90% alcohol, then 5 or 6 times with 10 ml portions of  $\text{NH}_4\text{Cl}$  soln, 2.40(a). Again wash well 6 or 8 times with 20 ml portions of 90% alcohol.

Dry ca 30 min. in  $100^\circ$  oven, cool, and weigh. Wash the  $\text{K}_2\text{PtCl}_6$  thru the Gooch with hot  $\text{H}_2\text{O}$ , using slight suction; then wash the Gooch with alcohol, dry, cool, and weigh. Difference in weight  $\times 0.1938 = \text{K}_2\text{O}$ . Report results as mg./100 g of original sample.

(b) *Short gravimetric chloroplatinate method.*—Proceed as directed under (a), pars. 3 and 4.

(c) *Short volumetric chloroplatinate method.*—Proceed as directed under (a), par. 3 only. Then dissolve precipitate in Gooch with several portions of boiling distilled  $\text{H}_2\text{O}$ . Stir gently to facilitate soln, and using suction collect filtrate and washings in 250 ml wide-mouthed, lipped Erlenmeyer flask. Add 1 ml of formic acid, heat to boiling, and simmer for ca 2 min. after metallic Pt has formed. Add 10 ml of  $\text{HNO}_3$  (1+1), mix, and then add small excess of 0.1  $N$   $\text{AgNO}_3$  accurately measured. Boil vigorously 5 min., cool, and filter thru Gooch crucible with fritted disk of medium porosity. Wash precipitate 5 or 6 times with 2%  $\text{HNO}_3$ , breaking up lumps with glass rod, and collect filtrate and washings in Erlenmeyer flask. Add 5 ml of saturated Fe alum indicator, 6.93(b), and with vigorous agitation titrate excess  $\text{AgNO}_3$  with 0.1  $N$   $\text{NH}_4\text{CNS}$  to first definite end point.

(d) *Long volumetric chloroplatinate method.*—Transfer the ash soln to round-bottomed porcelain dish of 100–200 ml capacity, using hot  $\text{H}_2\text{O}$ . If C or other insoluble material remains in the ash, filter into the dish thru 5.5 cm medium-textured paper, washing metal dish and filter 4 or 5 times with 5 ml portions of hot  $\text{H}_2\text{O}$ . Evaporate (rapidly if desired) to 10–15 ml, add the  $\text{H}_2\text{PtCl}_6$  soln in excess, and evaporate on steam bath to heavy consistency (impinge a stream of air on surface of liquid to hasten evaporation, and rotate dish from time to time to wash crystals into center). Cool dish, and if crystals become dry on cooling, add drop of  $\text{HCl}$  (1+4) and 2 drops of  $\text{H}_2\text{O}$ , so that mass remains moist and holds the salts in soln. Add ca 10 ml of 90% alcohol, triturate with policeman, and decant immediately onto prepared Gooch crucible. (15 ml Gooch crucible with rapid filtering mat, ca 3 mm thick, of acid-washed, long-fibered asbestos, to which is added 0.5–1 ml of  $\text{CaCO}_3$  suspension in such manner that the asbestos is completely covered when the liquid is removed by suction. The asbestos pad may be used for 3 or 4 determinations. The top surface,

impregnated with Pt powder, may be removed with sharp wire when filtering becomes too slow, and thin layer of asbestos may be added from time to time if pad becomes thin. Asbestos should be completely covered with layer of  $\text{CaCO}_3$  before each filtration.) Wash dish and crystals once or twice more with ca 5 ml portions of 90% alcohol, and then transfer precipitate to Gooch. Wash crucible free of  $\text{H}_2\text{PtCl}_6$  with 90% alcohol and then wash three or four times with 5 ml portions of  $\text{NH}_4\text{Cl}$  soln, 2.40(a), pouring soln gently into crucible from graduate so that  $\text{CaCO}_3$  mat is not disturbed. Wash the  $\text{NH}_4\text{Cl}$  from the crucible with 4 or 5 washings of 80% alcohol. Cover the  $\text{K}_2\text{PtCl}_6$  completely with the  $\text{Ca}(\text{CHO}_2)_2$  by adding ca 1 ml of the suspension. Remove liquid with suction and wash once with alcohol. Cover with  $\text{Na}_2\text{CO}_3$  to depth of 2–3 mm and moisten with ca 1 ml of alcoholic  $\text{NaOH}$ - $\text{NaCHO}_2$  soln. Ignite at ca  $500^\circ$  for 5–10 min. (If furnace is not available, ignition is conveniently conducted as follows: Prepare air bath by suspending nichrome triangle ca 1" from bottom of metal crucible, ca 2.5" diam. at top and 3" deep, and placing inverted porcelain crucible cover on the triangle. Heat over Meker burner with flame so adjusted that inside of the crucible is just red as far up as the suspended cover.)

Cool the Gooch, add ca 5 ml of hot  $\text{H}_2\text{O}$ , and filter by suction into 400–500 ml glass-stoppered Erlenmeyer flask. Repeat addition of hot  $\text{H}_2\text{O}$  once or twice to remove the  $\text{Na}_2\text{CO}_3$ . With suction on, add  $\text{HNO}_3$  (1+1) (ca 5 ml) dropwise to decompose the  $\text{CaCO}_3$  and  $\text{Na}_2\text{CO}_3$ . Wash several times with hot  $\text{H}_2\text{O}$ .

Add 10 ml of  $\text{HNO}_3$  (1+1) to the flask. Cool, and add 5 ml of Fe alum soln, 6.93(b), and quantity of 0.1 N  $\text{AgNO}_3$  soln, accurately measured, greater than that necessary to precipitate the Cl. Make to volume of ca 200 ml with  $\text{H}_2\text{O}$ , add 1–2 ml of nitrobenzene, stopper flask, and shake vigorously ca 30 seconds to coagulate the  $\text{AgCl}$ . Titrate excess  $\text{AgNO}_3$  with 0.1 N  $\text{NH}_4\text{CNS}$ . 1 ml of 0.1 N  $\text{AgNO}_3$  = 8.1 mg of  $\text{K}_2\text{PtCl}_6$ , 1.57 mg of  $\text{K}_2\text{O}$ , or 1.3 mg of K.

If approximate  $\text{AgNO}_3$  requirement is not known, following procedure is recommended: After adding the Fe alum soln, note reading on  $\text{NH}_4\text{CNS}$  buret and add a few drops to flask. Then, while swirling flask, run in 0.1 N  $\text{AgNO}_3$  from buret until soln becomes milk white, after which add an additional 1 or 2 ml. Continue determination as directed previously, beginning "Make to volume of ca 200 ml. . . ." Back titrate with the  $\text{NH}_4\text{CNS}$  soln and include the volume added previous to the addition of  $\text{AgNO}_3$  in calculating  $\text{AgNO}_3$  equivalent of sample.

#### Gravimetric Cobaltinitrite Method

##### 26.18

##### REAGENTS

(a) *Trisodium cobaltinitrite soln.*—Prepare an aqueous soln containing 2.0 g of  $\text{Na}_3\text{Co}(\text{NO}_2)_6$  in each 10 ml. Filter before use and prepare fresh soln before each set of determinations.

(b) *Nitric acid solns.*—Approximately 1 N and 0.01 N.

(c) *Nitric acid-dipotassium sodium cobaltinitrite wash soln.*—Saturate a portion of the 0.01 N  $\text{HNO}_3$  with a few mg of  $\text{K}_2\text{NaCo}(\text{NO}_2)_6 \cdot \text{H}_2\text{O}$  by shaking (ca an hour). Filter thru F Pyrex sintered-glass crucible or equivalent.

##### 26.19

##### DETERMINATION

Add enough 1 N  $\text{HNO}_3$  to the ash, 26.14(a) or (b), in Pt dish to yield excess of ca 2 ml of the acid in the 20 ml of soln used in precipitation (ca 3 ml for 26.14(a) and 5 ml for 26.14(b)). Wash into 25 ml volumetric flask, make to volume, and mix. Allow to stand at least 1 hour and filter, if necessary, thru small paper filter. Withdraw

10 or 20 ml aliquot (3–35 mg  $K_2O$ ), adjust to 20 ml if necessary, and cool to ca  $20^\circ$ .

Add from pipet while stirring 10 ml of the Na cobaltinitrite soln cooled to  $20^\circ$ . In the range 3–18 mg (most preserves) add the reagent dropwise with stirring; in the range 18–35 mg (most fruits) add the reagent in steady stream from fairly rapid delivery pipet (20–22 seconds). Allow to stand 2 hours at ca  $20^\circ$ . Protect precipitating vessel from laboratory fumes. Filter in tared sintered-glass filtering crucible (Pyrex F 35 ml capacity is convenient), using the cobaltinitrite wash soln to make transfer.

Wash precipitate nine times with at least 4 ml portions of this soln, once with 2 ml of 0.01  $N$   $HNO_3$ , and 5 times with 2 ml portions of alcohol, releasing vacuum each time before adding the washing fluid. Aspirate until dry. Dry for 1 hour at  $100^\circ$ , cool in desiccator, and weigh. Formula of precipitate is  $K_2NaCo(NO_2)_6 \cdot 11H_2O$ , and

$$\frac{\text{mg of precipitate} \times 0.2074 \times 100}{\text{g of sample in aliquot}} = \text{mg of } K_2O/100 \text{ g sample.}$$

#### NOTES:

(1) *Crucibles*.—Pyrex F or Jena 1G4 porosity are acceptable and can be used a number of times before cleaning with hot 5%  $H_2SO_4$ .

(2) *Washing of precipitate*.—The final wash with 0.01  $N$   $HNO_3$  should be restricted to 2 ml. The  $K_2NaCo(NO_2)_6$  precipitate obtained in K determinations is suitable for saturating the wash soln.

(3) *Control sample*.—Control sample of pure dry KCl should be run from time to time. Stock soln of 2 mg of  $K_2O$ /ml is convenient. Adjust to volume of 20 ml, using 2 ml of 1  $N$   $HNO_3$  for acidification. If recoveries are low, reagent should be rejected; if slightly high, blank correction may be made on the K estimations.

#### MANGANESE (8)—TENTATIVE

##### 26.20

##### PREPARATION OF SOLUTION

Dissolve the ash in HCl (1+2), evaporate to dryness, and heat at  $110^\circ$  for 1 hour to dehydrate any  $SiO_2$ . Dissolve residue in HCl (1+4) and filter into volumetric flask. Wash filter thoroly and make up to volume.

##### 26.21

##### DETERMINATION

To an aliquot of the prepared soln add sufficient Br water to oxidize any ferrous Fe to the ferric state. Boil off excess Br. Dilute to 150 ml and heat to boiling. Add sufficient 10%  $NaH_2PO_4$  soln to combine with all the Fe and Al. Add plenty of bromocresol green indicator, and while mixture is gently boiling add 10% freshly prepared NaOH soln dropwise to first permanent turbidity or an initial color change in event no Fe or Al compounds are present. Continue neutralization by slowly adding 20% Na acetate soln to give yellow-green color. Fe and Al phosphates are completely precipitated at pH of 4, at which point bromocresol green indicator is yellow-green (9). Boil gently 1–2 min. if any precipitate of Al or Fe phosphate forms. Allow to settle, filter, wash carefully, and discard precipitate. To filtrate add 10 ml of the Na acetate and adjust pH to 4.2–4.4 (indicated by a yellow-green color with bromocresol green indicator) by adding HCl (1+5) dropwise. Add sufficient Br water to color soln distinctly orange, cover with watch-glass, and boil gently ca 3 min. Take great care to avoid bumping. Allow mixture to settle, add a little more Br water, and again boil gently 1–2 min. Again allow to settle, filter, and wash beaker and filter thoroly. (The filtrate is reserved for Ca and Mg determinations.) Dissolve hydrated oxide precipitate from filter into original beaker with as little saturated  $SO_2$  soln as possible. Wash filter paper thoroly with hot  $H_2O$ . Boil to remove all odor of  $SO_2$ , add 10 ml of  $H_2SO_4$  and 10–20 ml of  $HNO_3$ , carefully dilute to 50–75 ml, and heat



to boiling, slowly introducing small quantities of  $\text{KIO}_4$  (ca 0.05 g) with spatula until maximum color is produced (ca 0.2 g of  $\text{KIO}_4$  is sufficient). Cool, and introduce into volumetric flask. The Mn in the final dilution for colorimetric comparison should be no more than 1 mg/50 ml. Compare color with standards prepared as directed in 12.15, except to substitute 10 ml of  $\text{HNO}_3$  for the  $\text{Fe}(\text{NO}_3)_3$ . Report as percentage of  $\text{Mn}_2\text{O}_3$  by multiplying  $\text{KMnO}_4$  by factor 0.4827.

#### CALCIUM (10)—TENTATIVE

##### 26.22

##### *Double Precipitation Method*

Evaporate filtrate from Mn determination, 26.21, to 100–150 ml. Boil off any Br remaining and adjust pH to 4.4–4.6 (green to green-blue with bromocresol green indicator) by adding 20% Na acetate soln (pH of 4.4–4.6 is most favorable for precipitation of Ca oxalate). Add sufficient saturated Na oxalate soln dropwise to precipitate all the Ca from the boiling soln, and continue to boil until oxalate begins to settle, or digest 15 min. on steam bath. Allow to settle until clear, filter, and wash precipitate thoroly with hot  $\text{H}_2\text{O}$ . Reserve filtrate and washings for Mg determination. Carefully wash precipitate back into original beaker, heat, and dissolve the oxalate by adding as little  $\text{HCl}$  as possible. Reprecipitate the Ca by adding  $\text{NH}_4\text{OH}$  soln (1+9) dropwise until pH is again 4.4–4.6 (green to green-blue with bromocresol green indicator). Add slight excess of saturated  $\text{NH}_4$  oxalate soln while still hot. Digest on steam bath 1 hour and set aside until supernatant liquid is clear, preferably overnight. Filter, and wash with hot  $\text{H}_2\text{O}$ . Determine the Ca either gravimetrically or volumetrically by the usual methods (for small quantities the gravimetric method is preferred). Report as  $\text{CaO}$ .

If Mg is not to be determined, precipitate the Ca once from the boiling soln (freed from Fe, Al, and Mn) with saturated  $\text{NH}_4$  oxalate soln, and proceed as directed above, beginning "Digest on steam bath 1 hour."

##### 26.23

##### *Single Precipitation Method*

Evaporate filtrate and washings from Mn determination, 26.21, to 200–250 ml. Add 8–10 drops of bromocresol green indicator and sufficient 20% Na acetate soln to change pH to 4.8–5.0 (blue). Cover with watch-glass and heat to boiling. Precipitate the Ca slowly by adding 3% oxalic acid soln, a drop every 3–5 seconds, until pH is changed back to 4.4–4.6 (optimum for Ca oxalate precipitation) as indicated by appearance of distinct green shade. Change of color will indicate excess of oxalic acid—more would develop yellow tints, showing an undesirable displacement of the pH. Boil 1–2 min. and allow mixture to settle until clear. Filter, and wash thoroly with hot  $\text{H}_2\text{O}$ . Determine either gravimetrically or volumetrically as directed in 26.22.

##### 26.24

#### MAGNESIUM (11)—TENTATIVE

Add 2–3 drops of  $\text{HCl}$  to filtrate and washings from Ca determination, 26.22, and evaporate to 75–100 ml. If quantity of phosphates naturally in sample, or added for purpose of precipitating Fe and Al, is insufficient to precipitate all the Mg expected, add more but avoid a large excess. For this purpose neutralize with 10%  $\text{NH}_4\text{OH}$  soln until permanent precipitate forms and add sufficient  $\text{NaH}_2\text{PO}_4$  soln to precipitate all Mg present. Dissolve precipitate by slowly adding 10%  $\text{HCl}$  dropwise. Use as little  $\text{HCl}$  as possible to obtain complete soln. Use considerable care and patience in next step because  $\text{MgHPO}_4$  begins to precipitate at pH of 6.7–6.8, which is the critical point. Heat soln to gentle boiling and add  $\text{NH}_4\text{OH}$  soln (1+9) at

rate of 4 drops a minute while maintaining a gentle boil until a crystalline precipitate commences to form. (The first precipitate must be crystalline, not gelatinous. If first precipitate is gelatinous, redissolve it with a little HCl and start precipitation again more slowly. Stirring assists crystallization, but sides of beaker should not be scratched.) After crystals have formed in considerable numbers hasten the precipitation. (This treatment gives crystalline  $\text{MgHPO}_4$ .) Continue addition of the dilute  $\text{NH}_4\text{OH}$  until soln is slightly ammoniacal. Allow mixture to cool slightly, then add  $\frac{1}{2}$  the volume of  $\text{NH}_4\text{OH}$  slowly and with constant stirring. Let stand until precipitate has been converted into  $\text{MgNH}_4\text{PO}_4$ , preferably overnight. Filter, and wash carefully with the dilute  $\text{NH}_4\text{OH}$ , until all chlorides have been removed. Dry, and ignite slowly until all the C is consumed. Cover, and ignite intensely. Weigh white  $\text{Mg}_2\text{P}_2\text{O}_7$  and report as  $\text{MgO}$ .  $\text{Mg}_2\text{P}_2\text{O}_7 \times 0.3621 = \text{MgO}$ . (Ignition of dark colored residues with a drop of 20%  $\text{NH}_4\text{NO}_3$  soln will often improve the color. If the nitrate is added, use care to avoid spattering.)

**26.25****ALCOHOL PRECIPITATE (d)—TENTATIVE**

To 100 ml of prepared soln, 26.2( $b_1$ ) or ( $c_1$ ), in beaker, add 4–8 g of sucrose (1 or 2 lumps of cube sugar) if sugar is not already present, and evaporate to volume of 20–25 ml. If water-insoluble matter separates during evaporation add more sugar. Cool to room temp. and add slowly and with constant stirring 200 ml of alcohol. Allow to stand at least 1 hour, filter on 15 cm qualitative paper, and wash precipitate with alcohol. Do not permit alcohol precipitate to dry before transferring it from paper. Wash precipitate back into original beaker with hot  $\text{H}_2\text{O}$ , rinsing filter paper thoroly. Evaporate soln to ca 20 ml and add 5 ml of HCl (1+2.5). If water-insoluble matter separates, stir well and, if necessary, warm slightly to dissolve. Again precipitate with 200 ml of alcohol, allow to stand 1 hour, and filter thru paper. Wash precipitate and paper thoroly with alcohol to remove all HCl. Rinse precipitate from filter paper into a Pt dish with hot  $\text{H}_2\text{O}$ , evaporate to dryness on steam bath, dry to constant weight in water oven, and weigh; ignite and weigh again. The loss in weight is the alcohol precipitate.

As the precipitate in many samples is colorless and almost invisible, care must be exercised that none is lost in the dissolving and transferring operations. If the quantity of the alcohol precipitate, as indicated by its volume in the first precipitation, is not excessive, the second filtration may be made thru a Gooch crucible containing a thin asbestos mat. If the alcohol precipitate is very pure and small in quantity it may not be visible at first. In this case, add a small amount of an electrolyte, like NaCl, which will flocculate the alcohol precipitate and render it visible.

**26.26****PECTIC ACID (d) (DI-GALACTURONIC ACID)—TENTATIVE**

Transfer 200 ml aliquot of prepared soln, 26.2( $b_1$ ) or ( $c_1$ ), to beaker, add 8–12 g of sucrose (2 or 3 lumps of cube sugar) if soln does not already contain sugar, and evaporate to ca 25 ml. If organic acids are to be determined in filtrate from the pectin, cool, add 3 ml of normal  $\text{H}_2\text{SO}_4$ , and immediately add with constant stirring 200 ml of alcohol, allow precipitate formed to settle, filter on 15 cm qualitative paper, and wash with alcohol. If organic acids are not to be determined, omit addition of  $\text{H}_2\text{SO}_4$ . Transfer precipitate to original beaker with hot  $\text{H}_2\text{O}$ , evaporate to ca 40 ml, and cool to 25° or below. If water-insoluble matter separates during evaporation, stir vigorously, and if necessary add a few drops of HCl (1+2.5), and warm; then cool again. Dilute 2–5 ml of 10% NaOH soln, depending upon volume of the precipitate, to 50 ml, and add to soln of the alcohol precipitate. Allow to stand 15 min., add 40 ml of  $\text{H}_2\text{O}$  and 10 ml of HCl (1+2.5), and boil 5 min. Filter, and

wash precipitate of pectic acid with hot  $H_2O$ . (This filtration should be rapid and the filtrate clear. If filtrate is cloudy or of colloidal nature, reject the determination. Colloidal filtrates are due to insufficient alkali or to saponification at too high a temp., or both. In such cases, repeat determination, using more alkali and keeping temp. low.) Wash precipitate of pectic acid back into beaker, adjust to volume of 40 ml, cool to below  $25^\circ$ , and repeat the saponification with the dilute  $NaOH$  soln, the precipitation with the dilute  $HCl$ , and the boiling as above described. Again filter and wash precipitate of pectic acid with hot  $H_2O$ , but only to point where test of filtrate shows negligible quantity of acid. (Not more than 500 ml of total filtrate should be necessary.) Wash the pectic acid into Pt dish and dry on steam bath and finally in water oven to constant weight. Weigh, ignite, and weigh again. The loss in weight is pectic acid.

**26.27****PROTEIN—OFFICIAL**

Proceed as directed under 2.24, 2.25, or 2.26, using 5 g of jelly or other fruit product containing a large quantity of sugar, or 10 g of juice or fresh fruit, and a larger quantity of the  $H_2SO_4$  if necessary for complete digestion. Percentage of  $N \times 6.25$  = percentage of protein.

**26.28****TITRATABLE ACIDITY***I. With Indicator—Official*

(a) *Colorless or slightly colored solns—Official.*—Dilute to ca 250 ml 10 g of prepared juice, 26.2(a), or 25 ml of prepared soln, 26.2(b)<sub>1</sub> or (c)<sub>1</sub>, with neutralized or recently boiled  $H_2O$ . Titrate with 0.1 N alkali, using 0.3 ml of 1% phenolphthalein soln for each 100 ml of soln being titrated. Report as ml of 0.1 N alkali/100 g or 100 ml of original material.

(b) *Highly colored solns—Tentative.*—Dilute sample of known weight with neutralized  $H_2O$  and titrate just below end point with 0.1 N alkali, using 0.3 ml of 1% phenolphthalein for each 100 ml of soln being titrated. Transfer measured quantity (2 or 3 ml) of this soln into ca 20 ml of neutral  $H_2O$  in small beaker. (In this extra dilution the color of the fruit juice becomes so pale that the phenolphthalein color is easily seen.) If test shows that end point has not been reached, pour extra diluted portion back into original soln, add more alkali, and continue titration to end point. By comparing dilutions in small beakers, differences produced by few drops of 0.1 N alkali can be easily observed.

*II. With Glass Electrode—Tentative***26.29****REAGENTS**

(a) *pH buffer of 0.05 M potassium acid phthalate (12).*—For use between 20 and  $25^\circ$ , lightly crush ca 12 g of  $KHC_8H_4O_4$  (Nat. Bur. Standards Sample 84b or equivalent) to fineness of ca 100-mesh and dry for 1–2 hours at  $120^\circ$ . Cool in desiccator containing good desiccant. Add 10.181 g to 1 liter of freshly boiled  $H_2O$ , pH 6.7–7.3, at  $25^\circ$ , or 10.193 g to 1 liter of  $H_2O$  at  $20^\circ$ . Set electrometer at pH 4.00 for operating temp.  $20$ – $22^\circ$ , pH 4.01 for the range  $23$ – $29^\circ$ .

(b) *pH buffer of sodium tetraborate and sodium chloride.*—Dissolve 3.814 g of recrystallized  $Na_2B_4O_7 \cdot 10H_2O$  and 1.169 g of reagent-grade  $NaCl$  in  $H_2O$  and make to 1 liter. (This soln has pH of 9.16 at  $25^\circ$  and 9.20 at  $20^\circ$ .)

**26.30****DETERMINATION**

Before use, check the electrometer and glass electrode with the standard buffer solns. Rinse glass electrode in  $H_2O$  several times until pH reading near 6 is obtained.

Also drain a few ml of saturated KCl soln from the bridge during the washing. Immerse electrode and bridge in sample contained in beaker. (Sample should titrate between 10 and 50 ml of 0.1 *N* NaOH and be contained in initial volume between 100 and 200 ml.) Stir moderately. Set electrometer for ca pH 6 and add the alkali quite rapidly until deflection of meter is small upon momentarily closing circuit. Set electrometer dial for pH 7 and add alkali slowly. After pH 7 is reached, finish titration by adding the 0.1 *N* alkali 4 drops at a time, and record total volume and pH reading after each addition. (Add whole drops, so that fraction of a drop is not remaining on buret tip.) Continue titration at least 4 drops beyond pH of 8.1, and interpolate data for titration corresponding to pH 8.1. The pH values used for interpolation should lie in range  $8.10 \pm 0.2$ .

NOTES:

- (1) Always keep glass electrode covered with H<sub>2</sub>O when not in use.
- (2) If strongly acid cleaning solns are used, a period of several hours is required for electrode to come to equilibrium on standing in H<sub>2</sub>O.
- (3) If electrode, bridge-arm, and stirrer are wiped lightly with piece of filter paper previous to insertion into the standard buffer, the same soln may be used for several checks on the instrument.
- (4) With some electrometers it is necessary to recheck the electrical balance with the standard cell frequently during extended titrations. Also the pH standardization should be checked after each of the first few determinations or until the batteries come to nearly constant rate of discharge, which is indicated when no further adjustment is necessary.
- (5) When unshielded leads are used on the electrode, an air-driven stirrer is necessary to eliminate electrical fields. Extension leads may be made of ordinary, insulated, braided copper wire.

26.31

VOLATILE ACIDS—OFFICIAL

Dissolve 10 g of the sample, dilute to 25 ml, and distil in current of steam as directed under 15.24. 1 ml of 0.1 *N* alkali = 0.0060 g of acetic acid.

TOTAL TARTARIC ACID (15)

*Bitartrate Method—Tentative*

26.32

PREPARATION OF SAMPLE

Choose a quantity of sample whose titratable acidity in terms of normal acid does not exceed 3 ml. Designate as "A" the ml of normal alkali required to neutralize quantity of sample chosen. In no case should solids content exceed 20 g (200 ml of sample soln of a jam or jelly). Adjust volume of sample to ca 35 ml either by evaporation or by the addition of H<sub>2</sub>O, add 3 ml of normal H<sub>2</sub>SO<sub>4</sub>, and heat to 50°. Pour adjusted sample into 250 ml volumetric flask, rinse with 10 ml of hot H<sub>2</sub>O and finally with alcohol, cool, dilute to mark with alcohol, shake, and filter thru folded paper (cover funnel with watch-glass). Pipet 200 ml of the filtrate into centrifuge bottle.

If sample contains alcohol, saponification is necessary. Adjust volume to 35 ml, add "A" + 3 ml of normal KOH, heat to ca 60°, and allow to stand overnight. Add "A" + 6 ml of normal H<sub>2</sub>SO<sub>4</sub> and transfer to 250 ml volumetric flask, as described previously. Filter, and pipet 200 ml of filtrate into centrifuge bottle.

26.33

DETERMINATION

To the soln in centrifuge bottle add quantity of Pb acetate soln, 26.36(d), equal to "A" + 3 ml, or in case saponification was made, "A" + 6. Shake vigorously for 2 min., and centrifuge at ca 1000 r.p.m. for 15 min. Carefully decant supernatant liquid from the precipitated Pb salts and test with small quantity of the Pb soln. If precipitate is formed, return mixture to centrifuge bottle, add more Pb soln, shake,

and again centrifuge. If sediment lifts, repeat centrifuging, increasing speed and time. Allow to drain thoroly by inverting bottle for several minutes. To material in centrifuge bottle add 200 ml of 80% alcohol, shake vigorously, and again centrifuge, decant, and drain. To the Pb salts in centrifuge bottle add ca 150 ml of  $H_2O$ , shake thoroly, and pass in  $H_2S$  to saturation. Transfer to 250 ml volumetric flask, dilute to mark with  $H_2O$ , and filter thru folded paper. Transfer 200 ml of clear filtrate to 400 ml beaker, and evaporate on gauze to 20 ml over small flame. Neutralize with normal potassium hydroxide, using phenolphthalein indicator, and add 5 drops of the alkali in excess. Add 2 ml of acetic acid and 80 ml of 95% alcohol slowly and with constant stirring. Chill in ice bath, stir vigorously 2 min., and place in refrigerator overnight. Decant supernatant liquid onto thin pad of asbestos in Gooch crucible with removable bottom, leaving ca 25 ml in beaker. To contents of beaker add ca 0.3 g of dry purified asbestos. Mix thoroly and wash into crucible with the cold filtrate. Finally wash beaker and crucible with 3 portions of 15 ml each of ice-cold 80% alcohol, sucking crucible dry each time. Transfer pad and precipitate to original beaker with ca 100 ml of hot  $H_2O$ , heat almost to boiling, and titrate with 0.1 *N* alkali, using phenolphthalein indicator. 1 ml of 0.1 *N* alkali = 0.015 g of tartaric acid

*Racemate Method (13) (Kling)—Tentative*

## 26.34

## REAGENTS

(a) *Diammonium-citrate soln.*—Dissolve 29 g of citric acid in ca 200 ml of  $H_2O$  and carefully neutralize with dilute  $NH_4OH$  soln, using methyl red indicator. Add 14.5 g of citric acid, dilute to 1 liter, and filter.

(b) *Ammonium-laevo-tartrate soln.*—Dissolve 3.2 g of ammonium-laevo-tartrate free of the dextro-modification in  $H_2O$ , dilute to 200 ml, and filter. Add 1 ml of formalin as a preservative.

(c) *Calcium acetate soln.*—Dissolve 16 g of  $CaCO_3$  in 120 ml of acetic acid diluted with  $H_2O$ , dilute to 1 liter, and filter.

(d) *Dilute hydrochloric acid soln.*—Dilute 34 ml of  $HCl$  with  $H_2O$  to 1 liter.

(e) *Calcium-sodium acetate soln.*—Dissolve 5 g of  $CaCO_3$  in 20 g of acetic acid, add 100 g of Na acetate, dilute to 1 liter, and filter.

(f) *Standard potassium permanganate soln.*—Dissolve 6.9745 g of purest  $KMnO_4$  in  $H_2O$  and dilute to 1 liter. Standardize soln against a soln of pure tartaric acid of known titer in same manner as in final titration. 1 ml of  $KMnO_4$  soln = nearly 0.005 g of tartaric acid.

(g) *Standard oxalic acid soln.*—Dissolve 13.8793 g of purest oxalic acid in  $H_2O$  and dilute to 1 liter. Titrate against the standard  $KMnO_4$  soln.

## 26.35

## DETERMINATION

Using the prepared sample, 26.32, proceed as directed under 26.33 thru the decomposition of the Pb salts with  $H_2S$ , dilution to 250 ml, and filtration. Pipet 200 ml of the clear filtrate into 400 ml beaker and evaporate to ca 100 ml. Add 50 ml of  $H_2O$ , 15 ml of the diammonium-citrate soln, 25 ml of the ammonium-laevo-tartrate soln, and 20 ml of the Ca acetate soln. Stir vigorously until Ca racemate begins to precipitate and allow to stand overnight at room temp. Decant onto thin, tightly-tamped pad of asbestos in Gooch crucible with removable bottom and transfer precipitate to Gooch with portion of the filtrate. Wash contents of crucible 5 times with  $H_2O$ , filling crucible about half full and sucking dry each time. Treat precipitate and mat, after removal from Gooch with 20 ml of the dilute  $HCl$  soln, and wash crucible thoroly with  $H_2O$ . Adjust volume of the soln to ca 150 ml with  $H_2O$ , add

50 ml of the Ca-Na acetate soln, and heat to ca 80°. Cool soln, stir vigorously, and allow to stand at least 4 hours, stirring occasionally. Filter, and wash as directed in the first operation. Transfer pad and precipitate to casserole with 150 ml of H<sub>2</sub>O, add 50 ml of H<sub>2</sub>SO<sub>4</sub> (1+9), and heat to 80°. Immediately add the standard KMnO<sub>4</sub> soln until excess is indicated. Again heat to 80°, add an additional 5 ml of the KMnO<sub>4</sub> soln, and allow to stand ca 1 min. After reheating to 80°, immediately add 10 ml of the standard oxalic acid soln and titrate back with the KMnO<sub>4</sub> soln. KMnO<sub>4</sub> soln required for oxidation (ml)  $\times 0.005 \div 2$  = tartaric acid in aliquot.

## CITRIC ACID (14)—TENTATIVE

## 26.36

## REAGENTS

- (a) *Potassium bromide soln.*—Dissolve 15 g of KBr in 40 ml of H<sub>2</sub>O.  
(b) *Potassium permanganate soln.*—Dissolve 5 g of KMnO<sub>4</sub> in H<sub>2</sub>O and dilute to 100 ml.  
(c) *Ferrous sulfate soln.*—Dissolve 40 g of FeSO<sub>4</sub>·7H<sub>2</sub>O in 100 ml of H<sub>2</sub>O containing 1 ml of H<sub>2</sub>SO<sub>4</sub>.  
(d) *Lead acetate soln.*—Dissolve 75 g of normal Pb acetate in H<sub>2</sub>O, add 1 ml of acetic acid, and dilute to 250 ml.

## 26.37

## DETERMINATION

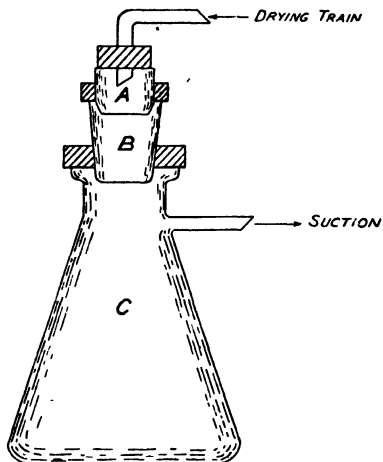
To the prepared soln, 26.32, in centrifuge bottle, add quantity of the Pb acetate soln equal to "A" + 3, or in case saponification was made, "A" + 6. Shake vigorously for 2 min. and centrifuge at ca 1000 r.p.m. for 15 min. Carefully decant supernatant liquid from precipitated Pb salts and test with small quantity of the Pb soln; if precipitate is formed, return to centrifuge bottle, add more Pb soln, shake, and again centrifuge. If sediment lifts, repeat centrifuging, increasing speed and time. Allow to drain thoroly by inverting bottle for several minutes. To material in centrifuge bottle add 200 ml of 80% alcohol, shake vigorously, and again centrifuge, decant, and drain. To the Pb salts in centrifuge bottle add ca 150 ml of H<sub>2</sub>O, shake thoroly, and pass in H<sub>2</sub>S to saturation. Transfer to a 250 ml volumetric flask, dilute to mark with H<sub>2</sub>O, and filter thru folded paper. Pipet 200 ml of filtrate into 500 ml Erlenmeyer flask, and evaporate to ca 75 ml. Cool, and add 10 ml of H<sub>2</sub>SO<sub>4</sub> (1+1) and 5 ml of the KBr soln. Heat mixture to 48–50°, allow to stand 5 min., and add 50 ml of the KMnO<sub>4</sub> soln. Mix, and allow to stand 1 min. Stopper flask, shake ca 1 min., and allow to stand 3 min. (During this time there should be a heavy deposit of MnO<sub>2</sub>; if necessary, add more KMnO<sub>4</sub> to assure excess of the oxidizing agent. If at any time during oxidation the precipitated MnO<sub>2</sub> disappears, discard determination and repeat, using more KMnO<sub>4</sub>.) Remove the MnO<sub>2</sub> with the FeSO<sub>4</sub> soln (ca 20 ml), cool to ca 15°, stopper flask, shake vigorously several minutes, and place in refrigerator overnight. Decant supernatant liquid onto thin, tightly tamped pad of asbestos in Gooch crucible (it is important that filtration be completed as quickly as possible). Note volume of filtrate (*S* in formula) and use filtrate to transfer precipitate to crucible. Wash contents of crucible at once with 50 ml of ice-cold H<sub>2</sub>O. Dry by aspirating with dry air or in vacuum desiccator and weigh. Remove pentabromacetone by treating contents of crucible with three portions of 20 ml each of alcohol and three portions of 20 ml each of ether. Again dry and weigh. Difference in two weights = weight of pentabromacetone. Calculate citric acid in aliquot by following formula:

$X = 0.445P + 0.018S$ , in which *X* = mg of citric acid in aliquot; *P* = weight of pentabromacetone (mg); and *S* = volume of filtrate (ml).

For drying the pentabromacetone by aspiration, use apparatus shown in Fig. 39.

- A, Gooch crucible (28 mm diam.) loosely packed with cotton;  
 B, Gooch crucible (35 mm diam.) for pentabromacetone; and  
 C, Suction flask (ca 500 ml capacity).

Dry the air by passing it thru  $\text{H}_2\text{SO}_4$  and soda-lime, and finally filter thru cotton. Cool the air entering drying train by passing it thru spiral condenser cooled with  $\text{H}_2\text{O}$ .



Allow crucible, B, containing the pentabromacetone, to remain under suction ca 1 min. to remove surface moisture before placing in apparatus. If air does not pass thru freely, place crucible in desiccator for short time. Maintain slow uniform flow of air by just "cracking" the suction. Dry until loss in weight does not exceed few tenths mg, making first weighing after 20 min.

#### LAEVO-MALIC ACID (1d)—TENTATIVE

(The method is empirical, therefore all the directions must be rigidly followed, particularly with respect to dilutions. The substitution of volumetric flasks of different capacities than those specified is not permissible.)

FIG. 39.—APPARATUS FOR DRYING PENTABROMACETONE BY ASPIRATION

#### 26.38

#### PREPARATION OF SAMPLE

Proceed as directed under 26.32, omitting addition of the 3 ml of normal  $\text{H}_2\text{SO}_4$  to adjusted sample. In case of saponification add "A" + 3 ml normal  $\text{H}_2\text{SO}_4$  to saponified material instead of "A" + 6 ml.

#### 26.39

#### REAGENTS

(a) *Lead acetate soln.*—Dissolve 40 g of normal Pb acetate in  $\text{H}_2\text{O}$ , add 0.5 ml of acetic acid, and dilute to 100 ml.

(b) *Standard tribasic lead acetate soln.*—Prepare soln from tribasic Pb acetate described below. To 5 g of the salt in 500 ml Erlenmeyer flask, add 200 ml of  $\text{H}_2\text{O}$  and shake vigorously. Neutralize 3 ml of normal  $\text{H}_2\text{SO}_4$ , diluted with 200 ml of  $\text{H}_2\text{O}$ , with the soln, using methyl red as indicator. Note volume of Pb soln required. In determination use 2 ml in excess of this quantity. (Soln should be freshly prepared.)

(c) *Tribasic lead acetate.*—Dissolve 82 g of normal Pb acetate in 170 ml of  $\text{H}_2\text{O}$ . Prepare 100 ml of dilute  $\text{NH}_4\text{OH}$  soln containing 5.8 g of  $\text{NH}_3$  as determined by titration (methyl red). Heat the solns to  $60^\circ$ , mix thoroly, and allow to stand overnight. Shake vigorously to break up precipitate, and filter on Büchner funnel; wash once with  $\text{H}_2\text{O}$  and suck dry, then twice with alcohol, and finally with ether. Allow to dry in air.

#### 26.40

#### DETERMINATION

To material in centrifuge bottle, add ca 75 mg of tartaric acid and quantity of the Pb acetate soln equal to "A" ("A" + 3 ml in case saponification was made),

shake vigorously 2 min. and centrifuge at ca 1000 r.p.m. for 15 min. Carefully decant supernatant liquid from precipitated Pb salts and test with small quantity of the Pb acetate soln. If precipitate is formed, return to centrifuge bottle, add more Pb acetate, shake, and again centrifuge. If the sediment lifts, repeat centrifuging, increasing speed and time. Allow to drain thoroly by inverting bottle for several minutes. Add ca 200 ml of 80% alcohol, shake vigorously, again centrifuge, decant, and drain. To Pb salts add ca 150 ml of  $H_2O$ , shake vigorously, and pass in rapid stream of  $H_2S$  to saturation. Stopper bottle and shake ca 1 min. Transfer to 250 ml volumetric flask with  $H_2O$ , dilute to mark, shake, and filter thru folded paper. Pipet 220 ml of the filtrate into 600 ml beaker and evaporate on gauze to ca 50 ml. Cool, neutralize with normal KOH (phenolphthalein), and add 5 drops in excess. Add 2 ml of acetic acid and transfer with alcohol to 250 ml volumetric flask. Add alcohol to mark, shake, and pour into 500 ml Erlenmeyer flask. Add small handful of glass beads and cool to 15°. Stopper flask, shake vigorously 10 min., and place in refrigerator for 30 min. Again shake 10 min. and filter thru a folded paper. Pipet 220 ml of clear filtrate into centrifuge bottle, add Pb acetate soln equal to "A" ("A" + 3 ml in case of saponification), shake vigorously ca 2 min., centrifuge, decant, and drain. Add 200 ml of 80% alcohol, shake, centrifuge, decant, and drain. Transfer the Pb salts to 500 ml Erlenmeyer flask with ca 175 ml of  $H_2O$ . Add 3 ml of normal  $H_2SO_4$ , heat to boiling, and add 1 ml of acetic acid (5+95) and the quantity of the standard tribasic Pb acetate soln previously determined, 26.39. Boil mixture 5 min., cool to room temp., transfer to 250 ml volumetric flask with  $H_2O$ , dilute to mark, shake, and pour into 500 ml Erlenmeyer flask. Add small handful of glass beads, cool to 15°, shake vigorously 5 min., and place in refrigerator for 30 min. Again shake 5 min. and filter thru folded paper. Saturate clear filtrate with  $H_2S$ , shake vigorously, and filter.

**Polarization.**—Evaporate 225 ml of the clear filtrate over gauze to ca 10 ml, neutralize with normal KOH (phenolphthalein), make slightly acid with the dilute acetic acid, and evaporate to ca 5 ml. Transfer to 25–27.5 ml Giles flask with  $H_2O$ , dilute to 27.5 ml mark, shake, and pour into small Erlenmeyer flask. If Giles flask is not available, use 25 ml measuring cylinder, dilute to mark, and add 2.5 ml of  $H_2O$  from buret. Add small handful of glass beads and 4 g of powdered uranyl acetate, shake vigorously for 10 min., and filter. (As the U-malic complex is sensitive to light, while shaking wrap flask in a towel and protect from light as much as possible during filtration and polarization.) Polarize in 200 mm tube at 20°, using white light. After filling tube, release tension on glass disks by slightly loosening caps, and allow to remain at 20° for at least 30 min. before making readings.

Ventzke reading  $\times$  factor 30.1 = mg of laevo-malic acid contained in the portion taken for analysis. If control for adjusting to standard temp. 20° is lacking, determine temp. of polariscope and at this temp. prepare the soln of the U complex as described above. Make readings after allowing tube to remain in trough of instrument 30 min.

#### INACTIVE MALIC ACID (16)—TENTATIVE

(The method is empirical, therefore all the directions must be rigidly followed, particularly with respect to dilutions. The substitution of volumetric flasks of different capacities than those specified is not permissible.)

#### 25.41

#### PREPARATION OF SAMPLE

Subject 2 portions of the sample to the isolation procedure, 26.43(a); use one portion for determination of laevo-malic acid (polarization) and the other for total malic acid, laevo + inactive (oxidation). Choose a quantity of sample that has a titratable acidity not exceeding 150 mg of acid calculated as malic acid. Designate as "A"



the ml of normal alkali required to neutralize the quantity of sample chosen. In no case should solids content exceed 20 g (200 ml of sample soln of a jam or jelly).

Adjust volume of sample to ca 35 ml either by evaporation or by addition of  $\text{H}_2\text{O}$ , pour into 250 ml volumetric flask, rinse with 10 ml of hot  $\text{H}_2\text{O}$  and then with alcohol, and dilute to mark with alcohol. Shake, and filter thru folded paper, draining thoroly and covering funnel with watch-glass. Pipet 225 ml of filtrate into centrifuge bottle.

## 26.42

### REAGENTS

Use reagents described under 26.39 and in addition—

(d) *Potassium permanganate soln.*—Dissolve 14.5214 g of purest  $\text{KMnO}_4$  in  $\text{H}_2\text{O}$  and dilute to 1 liter. Standardize soln as follows: Pipet 50 ml of the oxalic acid soln (e) into 600 ml beaker and add 70 ml of  $\text{H}_2\text{O}$  and 10 ml of  $\text{H}_2\text{SO}_4$  (1+1). Heat to  $80^\circ$ , immediately run in the  $\text{KMnO}_4$  soln until faint pink color is produced, again heat to  $80^\circ$ , and finish titration. Fifty ml of the  $\text{KMnO}_4$  soln should be equivalent to 50 ml of the oxalic acid soln; 1 ml of the oxalic acid soln  $\approx$  5 mg. of malic acid (laevo or inactive).

(e) *Oxalic acid soln.*—Dissolve 28.7556 g of purest  $\text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

## 26.43

### DETERMINATION

(a) *Isolation of total malic acid.*—To the soln in centrifuge bottle add ca 25 mg of citric acid and a quantity of the Pb acetate soln equal to "A" ("A" +3 ml in case of saponification), shake vigorously for 2 min., and centrifuge at ca 1000 r.p.m. for 15 min. Carefully decant supernatant liquid from the precipitated Pb salts and test with small quantity of the Pb acetate soln; if a precipitate is formed, return to centrifuge bottle, add more Pb acetate, shake, and again centrifuge. If sediment lifts, repeat centrifuging, increasing speed and time. Allow precipitate to drain thoroly by inverting bottle for several minutes. Add 200 ml of 80% alcohol and shake vigorously; again centrifuge, decant, and drain. Add ca 150 ml of  $\text{H}_2\text{O}$  to Pb salts, shake vigorously, and pass in rapid stream of  $\text{H}_2\text{S}$  to saturation. Stopper bottle and shake ca 1 min. Transfer mixture to 250 ml volumetric flask with  $\text{H}_2\text{O}$ , make to mark, shake, and filter. Pipet 225 ml of filtrate into 600 ml beaker, and evaporate to ca 100 ml to expel  $\text{H}_2\text{S}$ . Transfer to 250 ml volumetric flask with  $\text{H}_2\text{O}$ . (Volume in flask should be ca 200 ml.) Add 5 ml of acetic acid (1+9), and same quantity of Pb acetate soln previously used. Shake vigorously, dilute to mark with  $\text{H}_2\text{O}$ , and filter. Into the clear filtrate pass rapid stream of  $\text{H}_2\text{S}$  to saturation, stopper flask, shake vigorously, and filter. Pipet 225 ml of the filtrate into 600 ml beaker, add ca 75 mg of tartaric acid, and evaporate on gauze to ca 50 ml. Cool, neutralize with normal potassium hydroxide (phenolphthalein) and add 5 drops in excess. Add 2 ml of acetic acid and transfer mixture to 250 ml volumetric flask with alcohol. Dilute to mark with alcohol, shake, and pour into 500 ml Erlenmeyer flask. Add small handful of glass beads and cool to  $15^\circ$ . Stopper flask, shake vigorously for 10 min. and place in refrigerator for 30 min. Again shake 10 min. and filter thru folded paper. Adjust clear filtrate to  $20^\circ$  and pipet 225 ml into centrifuge bottle. Add Pb acetate soln equal to "A" ("A" +3 ml in case of saponification), shake vigorously ca 2 min., centrifuge, decant, and drain. Add 200 ml of 80% alcohol, shake, centrifuge, decant, and drain. Transfer the Pb salts to 500 ml Erlenmeyer flask with ca 175 ml of  $\text{H}_2\text{O}$ . Add 3 ml of normal  $\text{H}_2\text{SO}_4$  and heat to boiling; add 1 ml of acetic acid (5+95) and the quantity of the standard tribasic Pb acetate soln previously determined under 26.39(b). Boil mixture 5 min., cool to room temp., transfer to 250

ml volumetric flask with  $H_2O$ , make to mark, shake, and pour into 500 ml Erlenmeyer flask. Add small handful of glass beads, cool to ca  $15^\circ$ , shake vigorously 5 min., and place in refrigerator for 30 min. Again shake 5 min. and filter thru folded paper. Saturate clear filtrate with  $H_2S$ , shake vigorously, and filter. Use one of the two portions for polarization and the other for oxidation.

(b) *Polarization*.—Evaporate 225 ml of the clear soln over gauze to ca 10 ml and proceed as directed under polarization (laevo-malic acid), 26.40.

*Ventzke reading*  $\times$  factor 10.2 = mg of laevo-malic acid contained in the aliquot ("1" in the formula).

(c) *Oxidation*.—Evaporate 225 ml of the clear soln to ca 10 ml to expel last traces of alcohol, dilute to ca 120 ml with  $H_2O$ , and add 10 ml of 30% NaOH soln and 25 ml of the  $KMnO_4$  soln. Heat to ca  $80^\circ$  and place in boiling water bath for 30 min. Add 25 ml of the oxalic acid soln and 10 ml of  $H_2SO_4$  (1+1), stirring vigorously. Adjust temp. to  $80^\circ$ , and titrate to faint pink color with the  $KMnO_4$  soln. Again heat to  $80^\circ$  and finish titration. Quantity of  $KMnO_4$  soln used (ml)  $\times 5$  = total oxidizable material (as malic acid) present in aliquot ("t" in formula).

(d) *Calculation*.—Calculate the inactive malic acid "X" (mg) in portion taken for analysis by following formula:

$X = 4(t - 5 - l)$ , in which  $t$  = total oxidizable material (mg) as malic acid;  $l$  = laevo-malic acid (mg); 5 = correction factor for quantity of non-malic material (mg) as malic acid; and 4 = factor for reverting inactive malic acid in aliquot back to quantity of inactive acid in sample taken for analysis.

## 26.44

### LACTIC ACID (17)—OFFICIAL

Pipet 200 ml of prepared soln, 26.2 ( $c_1$ ), into 400 ml beaker and evaporate to 50 ml. Cool, and transfer contents of beaker to 250 ml volumetric flask with alcohol. Make to mark with alcohol, shake, and filter thru folded filter paper. Pipet 200 ml of filtrate into 400 ml beaker and evaporate to ca 25 ml. Add 50 ml of  $H_2O$  and again evaporate to 25 ml. Transfer material to continuous extractor (Fig. 30) with 25 ml of  $H_2O$  and proceed as directed under 22.12–22.13.

## 26.45

### VOLATILE FATTY ACIDS—OFFICIAL

(a) *Fruit juices (solids under 15%)*.—Weigh 100 g into distillation flask, Chap. 24, Fig. 37, and make to 150 ml with  $H_2O$ . Determine volatile acids as directed under 24.11. The mg of formic or acetic acid determined as present in distillation flask is that in 100 g of sample.

(b) *Fruit juices, jellies (solids 15% or more)*.—Weigh 100 g of prepared sample, 26.2(b), into 400 ml beaker, add ca 80 ml of  $H_2O$ , and warm, if necessary, until dissolved. Cool, and transfer with ca 20 ml of  $H_2O$  to continuous extractor of design similar to that used in determination of lactic acid, Chap. 22, Fig. 30, but having 200 ml capacity. (Outside tube of extractor is glass tubing 33–34 mm O.D., 115–120 cm in length with side arm 15–16 mm O.D., attached ca 63 cm from bottom. Inner tube is 12–13 mm O.D. flared at top to ca 25 mm diam.) Make distinctly acid to congo red paper with  $H_2SO_4$  (1+1) and extract with ether (free of volatile acids) until extraction is complete. (To establish time required for complete extraction, extract 200 ml of 0.01 N formic acid for 4 hours. Add 25 ml of 0.1 N NaOH to ether extract, swirl, and evaporate ether on steam bath. Titrate excess alkali with 0.1 N acid with phenolphthalein as indicator and calculate formic acid extracted. If all formic acid is not extracted, repeat extraction at 2 hour intervals until recovery is complete. Total time required to extract the formic acid is time necessary to assure complete extraction of volatile acids in determination.) Add ca 25 ml of  $H_2O$  to ether extract.

Add 5% NaOH soln in 5 ml portions until mixture is alkaline to phenolphthalein. Swirl after each addition of alkali. Add several glass beads and evaporate ether on steam bath, finally bringing aqueous soln to boiling on hot plate to remove last trace of ether. Transfer to distillation flask, using enough wash  $H_2O$  to make final weight 150 g. Make distinctly acid to congo red paper with  $H_2SO_4$  (1+1) and determine volatile acids as directed under 24.11. Mg of formic or acetic acid determined as present in distillation flask is that in 100 g of sample.

(c) *Frûits, jams, preserves*.—Weigh 2 portions of 100 g each of prepared sample, 26.2(c), into tared centrifuge bottles and make to 200 g with  $H_2O$ . Centrifuge, decant, and combine supernatant liquids. Transfer 200 g to extractor and proceed as directed under (b).

### PHOSPHORIC ACID

#### *Volumetric Method (18)—Official, First Action*

#### 26.46

#### REAGENTS

(a) *Molybdate soln*.—(1) Mix thoroly 50 g of  $MoO_3$  (99.5–100%) and 140 ml of  $H_2O$  and dissolve by addition of 72 ml of  $NH_4OH$  with stirring; (2) dissolve 50 g of powdered tartaric acid in 140 ml of  $H_2O$ ; (3) mix 295 ml of water-white  $HNO_3$  with 400 ml of  $H_2O$ . When solns are cool, pour soln (1) into soln (2) with stirring, and then pour the combined solns into soln (3). Keep in warm place (ca 40°) overnight, filter thru asbestos, and preserve in glass-stoppered bottle. When free from phosphates the soln is practically water-white.

(b) *Ammonium nitrate soln*.—Dissolve 500 g of  $NH_4NO_3$  in  $H_2O$  and dilute to 1 liter.

(c) *Carbon dioxide-free water*.—Recently boiled and cooled  $H_2O$ .

(d) *Standard sodium hydroxide and hydrochloric acid solns*.—0.1 *N*. Prepare as directed in 43.2–43.5 and 43.7–43.8.

(e) *Phenolphthalein indicator*.—1.0 g in 100 ml of alcohol.

#### 26.47

#### DETERMINATION

Dissolve the ash, 26.9, in 10–15 ml of  $H_2O$  and 3 or 4 ml of HCl and evaporate to dryness on steam bath. Take up in 10 ml of hot HCl (1+9) and transfer to 300 ml Erlenmeyer flask, keeping volume to 50–60 ml. (If  $P_2O_5$  is likely to exceed 10 mg, an aliquot should be taken.) Add 20 ml of the  $NH_4NO_3$  soln and heat in  $H_2O$  bath to 45–50°. Add 20 ml of the freshly filtered molybdate soln (this quantity will precipitate up to 20 mg of  $P_2O_5$ ) and allow flasks to remain in the bath at 45–50° for 30 min., mixing contents by swirling at ca 5 min. intervals. To prevent tipping, weight flasks with lead rings or by other means.

For filtration use filter-tube (so-called carbon filter), ca 28 mm inside diameter, fitted with removable, perforated porcelain disk from Caldwell crucible. (Caldwell or Gooch crucible can also be used.) Prepare quick filtering pad 2/16–3/16" thick, using short-fiber asbestos. For convenience in washing and in transferring filter tubes, provide suction flask with rubber stopper having hole somewhat larger than stem of filter tube.

With full suction, filter precipitate and wash flask and then filter tube ca 6 times each with cold  $H_2O$ , using 150–200 ml altogether. Test for complete washing by passing 25 ml of  $CO_2$ -free  $H_2O$  thru flask and filter tube into clean suction flask. Immediately disconnect suction and add 1 drop each of 0.1 *N* NaOH and phenolphthalein indicator, which should yield strong pink color.

Loosen pad and porcelain disk with wire or narrow rod inserted in stem end, and

transfer to flask. Place filter tube in neck of flask, dissolve any precipitate on walls with the standard alkali, and rinse down filter tube with ca 25 ml of the  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ . Add sufficient standard alkali to dissolve precipitate. Stopper flask, swirl, and allow to stand, mixing from time to time, until yellow precipitate is completely dissolved. Dilute to ca 75 ml with  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ , add 10 drops of phenolphthalein indicator, and titrate with the standard acid to complete disappearance of pink color, matching end point with another flask containing  $\text{H}_2\text{O}$  and asbestos only. Should the alkali adhere to fragments of asbestos, making end point uncertain, add slight excess of the acid and complete titration with the standard alkali. 1 ml of 0.1  $N$   $\text{NaOH}$  = 0.3088 mg of  $\text{P}_2\text{O}_5$ . Subtract for alkali consumed in a blank determination.

*Colorimetric Method (19)—Official*

**26.48**

REAGENTS

(a) *Molybdenum blue soln.*—Place 9.78 g of  $\text{MoO}_3$  (99.5–100%) in 500 ml Kjeldahl flask, add ca 150 ml of  $\text{H}_2\text{SO}_4$  (36  $N \pm 0.5 N$ ), and heat with gentle mixing until soln is complete. Cool to 150°. Weigh, on small watch-glass, 0.440 g of very finely powdered Mo metal (99.5–100%) and transfer to Kjeldahl flask by sliding watch-glass down neck of flask. Keep at 140–150° and mix vigorously until Mo is dissolved (some larger particles may remain). Cool, transfer to 250 ml volumetric flask, rinse the Kjeldahl with  $\text{H}_2\text{SO}_4$ , and transfer rinsings to the volumetric flask. Finally fill flask to 250 ml with  $\text{H}_2\text{SO}_4$  and mix well. Dilute 10 ml of this reagent with  $\text{H}_2\text{O}$  and titrate with 0.1  $N$   $\text{KMnO}_4$  to a pink color that persists for a minute. The reagent should be 0.11  $N \pm 0.001$ ; if less than 0.109  $N$  add a calculated quantity of Mo and dissolve by reheating in a Kjeldahl flask to 150°. Preserve the deep green soln in glass-stoppered bottles, carefully avoiding contamination of any kind.

(b) *Dilute molybdenum blue soln.*—Using a pipet previously wet inside with  $\text{H}_2\text{O}$ , pipet 10 ml of (a) into ca 60 ml of  $\text{H}_2\text{O}$  in a 100 ml volumetric flask. Wash pipet into flask, mix, cool, fill to mark with  $\text{H}_2\text{O}$ , and mix. As this reagent deteriorates with age, it should not be used 8 or 10 hours after preparation.

(c) *Sodium hydroxide soln.*—Phosphate and arsenate-free. 3.6  $N \pm 0.05 N$ . Should contain not over 0.0005%  $\text{PO}_4$ . Dissolve the  $\text{NaOH}$  in  $\text{H}_2\text{O}$ , using As-free Pyrex or porcelain vessel, cool, and titrate with standard acid. Preserve in paraffin-lined container. Avoid leaving glass equipment in contact with this reagent for any extended period.

(d) *Normal sodium hydroxide.*—From (c) prepare ca normal  $\text{NaOH}$ . Preserve in As-free Pyrex or paraffined container fitted with 1 hole stopper bearing Pyrex medicine dropper.

(e) *Normal sulfuric acid.*—Approximately.

(f) *Perchloric acid.*—60%.

(g) *Sodium alizarin sulfonate soln.*—Dissolve 0.20 g of Na alizarin monosulfonate in 100 ml of  $\text{H}_2\text{O}$  and filter. Preserve in indicator bottle.

(h) *Standard phosphate soln.*—0.05 mg  $\text{P}_2\text{O}_5$ /ml. Dissolve 0.1917 of pure dry  $\text{KH}_2\text{PO}_4$  in ca 200 ml of  $\text{H}_2\text{O}$ , and add 10 ml of normal  $\text{H}_2\text{SO}_4$  and 6 drops of 0.1  $N$   $\text{KMnO}_4$ . Dilute to exactly 2 liters. This soln keeps indefinitely in well-stoppered Pyrex bottle.

(i) *Glass beads.*—Boil a supply of small glass beads (2 or 3 mm in diam.) in aqua regia, wash clean with  $\text{H}_2\text{O}$ , and dry.

**26.49**

PREPARATION OF SAMPLE

Transfer a portion of sample containing 0.5–2.5 mg of  $\text{P}_2\text{O}_5$  to 500 ml Kjeldahl flask. (For determination of  $\text{P}_2\text{O}_5$  on water-soluble portion of fruits or fruit juices

25 or 30 ml (equivalent to 3.75 or 4.5 g of fruit) of the sample soln prepared as directed in 26.2(a) or (c<sub>1</sub>) is a convenient aliquot. For jams and jellies 50 ml of the prepared soln, 26.2(b<sub>1</sub>) or (c<sub>1</sub>) may be taken. If sample has low fruit content, a larger aliquot should be taken.) Add 5 ml of H<sub>2</sub>SO<sub>4</sub> from pipet or buret, then add 10 ml of HNO<sub>3</sub> and 5 or 6 small glass beads. Place flask on digestion rack over free flame. Protect flask from flame by asbestos mat having hole of such size that surface of H<sub>2</sub>SO<sub>4</sub> will be above mat. Boil over moderate flame until darkening begins (avoid excessive charring). Add a few ml of HNO<sub>3</sub> and again boil until slight darkening begins or until SO<sub>2</sub> fumes are evolved from a clear or amber soln. (In the case of jams or jellies, 3 or 4 additions (ca 5 ml each) of HNO<sub>3</sub> may be necessary.) Add to the hot flask 0.5 ml of the HClO<sub>4</sub> and continue fuming for a few minutes. (To avoid violent explosions of HClO<sub>4</sub> in the presence of organic matter do not add over 0.5 ml at one time and then only after practically all organic matter has been removed with HNO<sub>3</sub>, and do not fail to take all precautions advised in the use of HClO<sub>4</sub>.) When digest is water clear or very slightly greenish yellow, cool somewhat and cautiously add 50 ml of H<sub>2</sub>O and boil to fumes to remove traces of HNO<sub>3</sub>. Cool, add ca 25 ml of H<sub>2</sub>O, transfer to 100 ml volumetric flask, mix, cool, make to volume, and mix thoroly.

## 26.50

## DETERMINATION

Transfer a 20 ml aliquot of sample digest and 0, 2, 4, 6, 8, 10, and 12 ml of the standard phosphate soln to 100 ml volumetric flasks (Kohlrusch sugar flasks have been found convenient) marked at 70 ml capacity. To the standards add 30 ml of the normal H<sub>2</sub>SO<sub>4</sub>. To the samples add 20 or 25 ml of H<sub>2</sub>O, and to all flasks add 3 drops of the Na alizarin sulfonate soln and then exactly 10 ml of the 3.6 N NaOH soln. Adjust acidity to just yellow by means of the normal H<sub>2</sub>SO<sub>4</sub> and normal NaOH until a single drop of the normal H<sub>2</sub>SO<sub>4</sub> just changes color of the soln to yellow. Dilute to 70 ml and mix by swirling. Place the flasks in a boiling water bath and bring to that temp. With a pipet add exactly 10 ml of the dilute molybdenum blue reagent, directing the stream into the soln (do not allow it to run down side of flask), mix by swirling, and continue to heat in the boiling water bath for exactly 20 min. Cool rapidly in cold H<sub>2</sub>O, dilute to volume, and mix.

(Keep the standards and unknowns at the same temp. by immersing the flasks in a boiling water bath wherein the H<sub>2</sub>O comes above level of soln in flask. A simple water bath may be prepared by placing a  $\frac{3}{4}$ " mesh wire screen in bottom of a 12 or 14" granite pan and filling pan with H<sub>2</sub>O to such a depth that liquid in flasks will be below level of H<sub>2</sub>O. Place pan on a stand and heat with a large Meker burner with flame so adjusted that it spreads over bottom of pan and keeps entire content at a gentle rolling boil. Place the flasks only around periphery of the pan and weight with lead rings or otherwise support to prevent tipping. Keep the bath at a rolling boil thruout heating period and add *boiling* H<sub>2</sub>O to the bath from time to time to keep level of H<sub>2</sub>O above level of liquid in flasks. Keep a thermometer in the bath and do not permit a variation of more than 2° between the center and the edge of the pan.)

Determine color intensity by means of a neutral wedge photometer (20), using a 1" cell, No. 66 filter, with Jena 0-2 neutral wedge. (Filter 66 is 4.5 mm Corning dark pyrometer red No. 241. With "B & L. Smoke C" glass wedge, use filter 65. Filter 65 is the same as 66 plus a half mm of Jena BG18.)

The method covers a range up to 0.6 mg of P<sub>2</sub>O<sub>5</sub> in the final 100 ml of soln. Make a large scale graph of the standards, plotting mg of P<sub>2</sub>O<sub>5</sub> against photometer readings. (Graph paper 20×36" with 10 lines to the inch is convenient.) By means of this plot convert the sample photometer readings to mg of P<sub>2</sub>O<sub>5</sub> present in final 100 ml of soln. If preferred, the equation of the line may be calculated as described by Klein and Vorhes (21) and the equation used in conversion.

## NOTES

The photometer need be calibrated but once for each batch of reagents provided the adjustment is not altered and the temp. of the boiling water bath remains the same. It is advisable, however, to develop one or two standards with each batch of unknowns in order to detect any possible change of conditions.

It will be noted that standardization under these conditions automatically corrects for the blank on reagents, except  $\text{HNO}_3$  and  $\text{HClO}_4$ . These reagents have not been found to contain significant quantities of As or P. It is well, however, to determine the digestion blank on these reagents from time to time.

In the analysis of heterogeneous samples, such as lots of fresh fruit, for total  $\text{P}_2\text{O}_5$ , it may be necessary to digest a larger portion than specified above in order to minimize sampling and weighing error. In that case it is convenient to take double the above sample and double the amount of  $\text{H}_2\text{SO}_4$  (10 ml.). Make digest to 200 ml, and finally transfer a 20 ml aliquot to 100 ml volumetric flask for color development. Amount of sample digested may be varied to suit nature of the sample if final aliquot taken for color development contains not more than 1 ml of  $\text{H}_2\text{SO}_4$  and not more than 0.6 mg of  $\text{P}_2\text{O}_5$ .

Fe, nitrate, and As act as interferences in development of color. Nitrates are not present in solns prepared as described, and neither Fe nor As is ordinarily present in fruit or fruit products in sufficient quantity to constitute an interference. However, if presence of excessive As or Fe is suspected, their interference may be prevented by a procedure used by Zinzadze (22). Proceed as directed previously to the point, "Adjust acidity to just yellow," after which add 10 ml of exactly normal  $\text{H}_2\text{SO}_4$  and then 10 ml of 8%  $\text{Na}_2\text{SO}_3$  and dilute to 70 ml. Heat in a boiling water bath for one hour. Then again refer to previous directions and continue with "add exactly 10 ml of the dilute molybdenum blue reagent." Standards and blank, of course, must then be treated in exactly the same manner.

**26.51 FREE MINERAL ACIDS—TENTATIVE.—See 33.85–33.87**

**SUCROSE**

**26.52 By Polarization—Official**

Determine by polarizing before and after inversion, as directed under 34.23, 34.24, or 34.29.

**26.53 By Reducing Sugars Before and After Inversion—Official.—See 34.30**

**26.54 REDUCING SUGARS—OFFICIAL.—See 34.39. Express results as invert sugar**

**26.55 COMMERCIAL GLUCOSE—OFFICIAL.—See 34.32**

**26.56 DEXTRIN—TENTATIVE**

Dissolve 10 g of sample in 100 ml flask and add 20 mg of KF and then ca  $\frac{1}{4}$  cake of compressed yeast. Allow fermentation to proceed below  $25^\circ$  for 2–3 hours to prevent excessive foaming and then incubate at  $27\text{--}30^\circ$  for 5 days. Clarify soln with basic Pb acetate soln and alumina cream; make up to 100 ml, filter, and polarize in 200 mm tube. A pure fruit jelly will show a dextro or laevo rotation of not more than a few tenths of a degree. If polariscope having Ventzke scale is used and 10% soln is polarized in 200 mm tube, number of degrees read on sugar scale of the instrument  $\times 0.8755$  = percentage of dextrin; or the following formula may be used:

$$\text{Percentage of dextrin} = \frac{C \times 100}{198 \times L \times W}, \text{ in which}$$

$C$  = degrees of circular rotation;  $L$  = length of tube in decimeters; and  $W$  = weight of sample in 1 ml of soln.

## STARCH

26.57

*Qualitative Test—Official*

Dilute a portion of sample with  $H_2O$ , heat nearly to boiling, add several ml of  $H_2SO_4$  (1+9), and then 10%  $KMnO_4$  soln until all color is destroyed. Cool, and test with I soln, 33.29(f). (Presence of starch is not necessarily indication of its addition as adulterant. It is usually present in small quantity in the apple, and occasionally in other fruits, and unless it is found in the fruit product in considerable quantity its presence may be due to these natural sources.)

## GELATIN (25)

26.58

*Qualitative Test—Tentative*

Gelatin in jellies and jams is shown by increased content of N. Precipitate a concentrated soln of jelly or jam with 10 volumes of absolute alcohol and determine N in dried precipitate as directed under 2.24, 2.25, or 2.26.

## AGAR AGAR

26.59

*Detection by Microscopic Examination (24)—Tentative*

Heat the jelly with  $H_2SO_4$  (1+18), add a crystal of  $KMnO_4$ , and allow to settle. If agar agar is present, the sediment will be rich in diatoms, which can be detected under microscope. (The diatoms adhere to the glass and are best obtained by pouring out the liquid, washing glass with 2 or 3 drops of alcohol, and transferring alcohol to microscopic slide by means of glass rod.)

26.60

*Detection by Precipitation—Tentative*

Cover 30 g of the jam or jelly with 270 ml of hot  $H_2O$ , stir until thoroly disintegrated, and boil 3 min. Filter soln while boiling hot thru rapid qualitative filter paper. In presence of agar agar a precipitate will form upon standing not longer than 24 hours. Filter, wash with cold  $H_2O$ , and dissolve from paper by means of very small quantity of boiling  $H_2O$ . Upon chilling this soln a firm jelly that can be examined by the touch will be formed. This method will detect 0.2% of agar agar with certainty if proportions of jam or jelly and  $H_2O$  specified are strictly followed.

26.61

## ADDED WATER IN GRAPE JUICE (26)—TENTATIVE

(Applicable to white juices only)

Measure ca 50 ml of *filtered* juice into 2 oz. tincture bottle containing a number of short pieces of glass rod. Add ca 1 g of finely powdered purest K acid tartrate, cool to 25°, and shake 1 hour at this temp. (There should be undissolved bitartrate in the juice; if there is not, repeat operation, using more of the salt.) *Immediately* filter juice and titrate 10 ml of filtrate with 0.1 N alkali, using phenolphthalein indicator. In same manner titrate 10 ml of the original filtered juice. An increase in titer of treated sample is index of added  $H_2O$ . Make the two titrations side by side in order to obtain same shade of pink.

For control of temp. during saturation period the following procedure is suggested: Immerse the tightly corked tincture bottle, neck down, into pint Mason jar filled top-full with  $H_2O$  at 25°. Adjust cover, immerse jar in pail of  $H_2O$  at 25° and maintain this temp. for 30 min. Remove jar from  $H_2O$  and immediately wrap in 3 sheets of heavy paper, making each wrapping separately. Place the system in

shaker and shake for 1 hour. Ascertain temp ( $t^\circ$  in formula) of the  $H_2O$  in the jar. Determine titers of treated and untreated juices as directed above and calculate volume % of added sugar soln ( $H_2O$ ) by following formula:

$W = 3.13(b-a) - 15.8 - 2.08(t^\circ - 25)$ , in which  $W$  = vol. % added  $H_2O$  (20% sugar soln);  $b$  = acidity of treated juice (ml 0.1  $N$  alkali/100 ml);  $a$  = acidity of original juice (ml 0.1  $N$  alkali/100 ml); and  $t^\circ$  = temp. of  $H_2O$  in Mason jar after shaking.

[Pure factory juices examined by this method show a small quantity of added  $H_2O$  (1-3%).]

26.62

METALS.—See Chap. 29

26.63

PRESERVATIVES.—See Chap. 32

26.64

COLORING MATTERS.—See Chap. 21

26.65

SWEETENING SUBSTITUTES.—See 32.11-32.16, 32.37-32.38

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- (6) Ibid., **11**, 216 (1928).
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- (11) Ibid., **14**, 473 (1931).
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- (15) Ibid., **15**, 648 (1932).
- (16) Ibid., **16**, 281 (1933).
- (17) Ibid., **20**, 605 (1937); **26**, 199 (1943).
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## 27. GRAIN AND STOCK FEEDS

### 27.1

#### SAMPLING (1)—OFFICIAL, FIRST ACTION

Insert sharp, closed-end sampler horizontally into package. Take cores from not less than 10% of bags present unless this process necessitates cores from more than 20 bags, in which case take core from 1 bag for each additional ton represented. If less than 200 bags, sample not less than 20 bags; if less than 20 bags, sample all bags. Thoroughly mix portions taken on clean oilcloth or paper, reduce by quartering to quantity of sample required, and place in air-tight container.

### 27.2

#### PREPARATION OF SAMPLE—OFFICIAL

Grind sample to pass thru sieve having circular openings  $1/25''$  (1 mm) in diam. and mix thoroughly. If sample cannot be ground, reduce to as fine a condition as possible.

#### 4 MOISTURE (2)

### 27.3

#### I. Drying with Heat—Official

Dry to constant weight at  $95-100^{\circ}$  under pressure not to exceed 100 mm of Hg (ca 5 hours), a quantity of the substance representing ca 2 g of dry material. Use covered Al dish at least 50 mm in diameter and not exceeding 40 mm in depth. Report loss in weight as moisture.

#### II. By Distillation with Toluene—Official

### 27.4

#### APPARATUS

A 250 ml distilling flask of Pyrex or other resistant glass connected by means of "distilling tube receiver" to 20" sealed-in, straight-tube Liebig condenser with delivery tube not over  $5/16''$  in diam. in manner shown, Fig. 40. Receiver, dimensions shown, is made by attaching proper side tube to calibrated section of 5 ml Mohr pipet and sealing outlet. Tube is calibrated in ml by distilling known quantities of  $H_2O$  into the graduated column, and the column of  $H_2O$  may be read to hundredths with reasonable accuracy. Clean tube and condenser with  $K_2Cr_2O_7-H_2SO_4$  mixture, rinse thoroughly with  $H_2O$ , then with alcohol, and dry in oven to prevent undue quantity of  $H_2O$  adhering to inner surfaces during determination.

### 27.5

#### DETERMINATION

If sample is likely to bump, add enough dry sand to cover bottom of flask. Add sufficient toluene to cover sample completely (ca 75 ml). Weigh and introduce into toluene sufficient sample to give 2-5 ml of  $H_2O$  and connect apparatus as shown, Fig. 40. Fill receiving tube with toluene, pouring it thru top of condenser. Bring to boil and distil slowly, ca 2 drops per second, until most of  $H_2O$  has passed over; then increase rate of distillation to ca 4 drops/second. When all  $H_2O$  is apparently over, wash down condenser by pouring toluene in at top, continuing distillation a short time to ascertain whether any more  $H_2O$  will distil over; if it does, repeat washing down process. If any  $H_2O$  remains in condenser, remove it by brushing down with tube brush attached to Cu

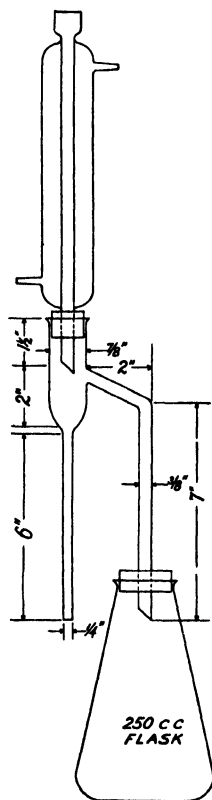


FIG. 40.—APPARATUS USED IN METHOD II FOR DETERMINATION OF MOISTURE

wire and saturated with toluene, washing down condenser at same time. (Entire process is usually completed within 1 hour.) Allow receiving tube to come to room temp. If any drops adhere to sides of tube, force them down by means of Cu wire with end wrapped in rubber band. Read volume of  $H_2O$  and calculate to percentage.

### III. *Drying without Heat over Sulfuric Acid (3)*—Official

27.6

#### REAGENT

*Sulfuric acid.*—Boil  $H_2SO_4$  in large Kjeldahl flask for 4 hours, close mouth of flask with stopper carrying  $CaCl_2$  tube, and cool.

27.7

#### DETERMINATION

Weigh suitable quantity of sample (2–5 g) into metal dish 5–10 cm in diam. and provided with tightly fitted cover. (If subsequent fat determinations are to be made, fat extraction cones may be used.) Mix substances that dry down to horn-like material with fat-free cotton or other suitable material. Place 200 ml of the fresh  $H_2SO_4$  in strong, tight vacuum desiccator. Place dish, uncovered, in desiccator and exhaust by means of vacuum pump to pressure of not more than 10 mm of Hg.

If pump is not available, place 10 ml of ether in small beaker in desiccator and exhaust with water filter pump. Between pump and desiccator interpose empty bottle next to desiccator and bottle of  $H_2O$  next to pump. Draw air from desiccator thru the  $H_2O$  and turn desiccator stopcock the instant the  $H_2O$  begins to rise in tube leading from empty bottle.

Gently rotate desiccator 4 or 5 times during first 12 hours. At end of 24 hours open desiccator, causing incoming air to bubble thru  $H_2SO_4$ , place cover on dish, and make first weighing. After weighing place sample in desiccator containing fresh  $H_2SO_4$  and exhaust as before. Rotate desiccator several times during the interval and weigh again after suitable period of drying. Repeat this process until weight is constant.

27.8

### IV. *Electric Air-Oven Method (4)*—Official

(Not intended for use when subsequent fat determination is to be made on same sample.)

Regulate electric air oven to  $135^\circ \pm 2^\circ$ . Using low, covered Al dishes, 27.3, weigh ca 2 g of sample into each dish and shake until contents are evenly distributed. With covers removed, place dishes and covers in oven as quickly as possible and dry samples for 2 hours. After placing covers on dishes transfer them to desiccator to cool. Weigh, and calculate loss in weight as moisture.

27.9

#### ASH (5)—OFFICIAL

Weigh 2 g sample into porcelain crucible and place in muffle furnace previously heated to  $600^\circ$ . Maintain at this temp. 2 hours with automatic control pyrometer. Transfer crucibles directly to desiccator, cool, and weigh immediately. Report percentage to first decimal place.

27.10

#### CRUDE PROTEIN—OFFICIAL

Determine N as directed under 2.24, 2.25, or 2.26, and multiply result by 6.25.

#### QUALITATIVE TESTS FOR PROTEINS (6)—OFFICIAL

*Biuret Test*—Unreliable in presence of glycerol

27.11

#### REAGENT

Add slowly with stirring 25 ml of 3%  $CuSO_4 \cdot 5H_2O$  soln to 1 liter of 10% NaOH. If it is necessary to filter reagent, use glass wool.

## 27.12

## DETERMINATION

To 2 or 3 ml of protein soln add, with shaking, a few drops of reagent. If characteristic pink or violet color does not develop quickly, allow soln to stand 15 or 20 min. In presence of  $(\text{NH}_4)_2\text{SO}_4$  the addition of NaOH is necessary.

(a) *Osborne modification.*—This modification of biuret test greatly increases its delicacy. Make the test as described above. Then add 10–20 drops of alcohol and a piece of solid NaOH (ca 5 g). The alkali “salts out” the small quantity of alcohol, which carries with it the color present, and in this way the presence of small quantities of protein can be detected.

The biuret test is dependent on the peptide grouping,  $-\text{HN}.\text{CO}.\text{NH}-$ , and therefore is given by all proteins. It is also given by certain other compounds containing similar groupings, such as biuret,  $\text{H}_2\text{N}.\text{CO}.\text{NH}.\text{CO}.\text{NH}_2$ , and malonamide,  $\text{H}_2\text{N}.\text{CO}.\text{CH}_2.\text{CO}.\text{NH}_2$ . Compounds containing one  $-\text{CO}.\text{NH}_2$  and one  $-\text{CSNH}_2$ ,  $-\text{C}(\text{NH})\text{NH}_2$  or  $-\text{CH}_2\text{NH}_2$  similarly joined will also respond to this test.

*Millon Test*

(Given by all aromatic substances, such as phenol and salicylic acid, which contain a benzene nucleus with substituted hydroxyl group. In proteins this grouping is furnished by the amino acid tyrosine.)

## 27.13

## REAGENT

Dissolve, by gently warming, one part by weight of Hg in two parts by weight of  $\text{HNO}_3$ , sp. gr. 1.42. Dilute soln with two volumes of  $\text{H}_2\text{O}$ . Allow mixture to stand overnight and decant supernatant liquid. The soln contains  $\text{Hg}(\text{NO}_3)_2$  and  $\text{HgNO}_3$ ,  $\text{HNO}_3$ , and some  $\text{HNO}_2$ .

## 27.14

## TEST

Add a few drops of reagent to 4 or 5 ml of protein soln in test tube. Warm gently by immersing a few minutes in hot  $\text{H}_2\text{O}$ . Pink or red color slowly develops and precipitate usually forms. If substance is a solid, suspend in 3 or 4 ml of  $\text{H}_2\text{O}$  and treat as directed above. Alkaline solns should first be neutralized to avoid precipitation of  $\text{HgO}$ .

*Glyoxylic Acid Test (Hopkins-Cole)*

## 27.15

## REAGENT

Add sufficient  $\text{H}_2\text{O}$  to cover liberally 10 g of powdered Mg in large Erlenmeyer flask. Add 250 ml of cold saturated oxalic acid soln, keeping flask cool under water tap during addition of acid. After reaction is over, shake mixture and filter. Acidify filtrate with acetic acid and make volume up to 1 liter with  $\text{H}_2\text{O}$ .

## 27.16

## TEST

To 1 or 2 ml of protein soln in test tube add 3 ml of reagent and mix thoroly. By means of pipet allow mixture to flow gently down side of second test tube (slightly inclined) containing 5 ml of  $\text{H}_2\text{SO}_4$ . Reddish-violet color forms at junction of fluids, owing to presence of tryptophane in the protein.

## 27.17

*Adamkiewicz Test*

Proceed as directed under 27.16, except to use acetic acid instead of a prepared soln of glyoxylic acid. Color reaction depends on presence of traces of glyoxylic acid formed from the acetic acid.

## 27.18

*Xanthoproteic Test*

Add ca 1 ml of  $\text{HNO}_3$  to 3 ml of the protein soln. White precipitate forms, which on boiling assumes a yellow color and may dissolve to give a yellow soln. Cool, and make slightly alkaline by careful addition of 30%  $\text{NaOH}$  soln. Color changes to deep orange. Color development depends on formation of nitro derivatives attached to benzene nucleus, and in proteins is referable primarily to the amino acids tyrosine and phenylalanine.

## ALBUMINOID NITROGEN—OFFICIAL

## 27.19

## REAGENT

*Cupric hydroxide*.—Dissolve 100 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 5 liters of  $\text{H}_2\text{O}$ ; add 2.5 ml of glycerol, and then add 10%  $\text{NaOH}$  soln until liquid is slightly alkaline; filter; rub precipitate in mortar with  $\text{H}_2\text{O}$  containing 5 ml of glycerol/liter; and wash by decantation or filtration until washings are no longer alkaline. Again rub precipitate in mortar with  $\text{H}_2\text{O}$  containing 10% of glycerol, thus preparing a uniform gelatinous mass that can be measured with pipet. Determine approximately quantity of  $\text{Cu}(\text{OH})_2$  in 5 ml by diluting to 50 ml with  $\text{H}_2\text{O}$ , filtering, washing, igniting, and weighing as  $\text{CuO}$ .

## 27.20

## DETERMINATION

Place 0.7 g of sample in beaker, add 100 ml of  $\text{H}_2\text{O}$ , and heat to boiling; or, in case of substances rich in starch, heat on steam bath for 10 min.; add quantity of the reagent that contains ca 0.5 g of the  $\text{Cu}(\text{OH})_2$ , stir thoroly, filter when cold, wash with cold  $\text{H}_2\text{O}$ , and without removing precipitate from filter determine N as directed under 2.24, 2.25, or 2.26, adding sufficient  $\text{K}_2\text{S}$  or  $\text{Na}_2\text{S}$  soln, 2.22(f), to precipitate all the Cu and Hg. Filter paper used must be practically free from N. If the material (such as seeds, seed residue, or oil cake) is rich in alkaline phosphates, add 1–2 ml of 10% soln of  $\text{NH}_3$ -free Na alum to decompose alkaline phosphates, then the  $\text{Cu}(\text{OH})_2$ , and mix well by stirring. If this is not done,  $\text{Cu}_3(\text{PO}_4)_2$  and free alkali may be formed and the protein-Cu precipitate partially dissolved in the alkaline liquid.

## 27.21

## AMIDO NITROGEN—OFFICIAL

Subtract percentage of albuminoid N from percentage of total N to obtain amido N.

## UREA AND AMMONIACAL NITROGEN (7)—OFFICIAL

## 27.22

## REAGENTS

(a) *Standard acid*.—See 2.22(a) or (b).

(b) *Standard alkali*.—See 2.22(c).

(c) *Indicator*.—See 2.22(h) or (i).

(d) *Defoaming soln*.—Dissolve 50 g of diglycol stearate in 375 ml of benzene, 75 ml of alcohol, and 250 ml of dibutyl phthalate, with warming if necessary.

(e) *Urease soln*.—Prepare fresh soln by dissolving standardized urease in  $\text{H}_2\text{O}$  so that each 10 ml of neutralized soln will convert the N of at least 0.1 g of pure urea. *Suggested standardization procedure*.—To determine alkalinity of commercial urease preparation dissolve 0.1 g in 50 ml of  $\text{H}_2\text{O}$  and titrate with 0.1 *N*  $\text{HCl}$ , using methyl red indicator. Add this quantity of 0.1 *N*  $\text{HCl}$  to each 0.1 g of urease in preparing the urease soln. To determine the enzyme activity prepare ca 50 ml of a neutralized 1% soln. Add different quantities of soln to 0.1 g samples of pure urea and follow with the enzyme digestion and distillation as directed in the determina-

tion. Calculate activity of the urease preparation from quantity of this urease soln that converted the urea, thereby permitting complete recovery of the N by distillation.

(f) *Calcium chloride soln.*—Dissolve 25 g of  $\text{CaCl}_2$  in 100 ml of  $\text{H}_2\text{O}$ .

## 27.23

## DETERMINATION

Place 2 g of sample in Kjeldahl flask with ca 250 ml of  $\text{H}_2\text{O}$ . Add 10 ml of the urease soln, stopper tightly, and let stand at room temp. 1 hour or at  $40^\circ$  for 20 min. Cool to room temp. if necessary. Use more urease soln if feed contains more than 5% urea (ca 12% protein equivalent). Rinse stopper and neck with a few ml of  $\text{H}_2\text{O}$ . Add 2 g or more of  $\text{MgO}$  (heavy type), 1 ml of the  $\text{CaCl}_2$  soln, and 5 ml of the defoamer soln, and connect flask with condenser by means of Kjeldahl connecting bulb. Distil 100 ml into measured quantity of the standard acid, and titrate with the standard alkali, using cochineal or methyl red indicator.

## C. FAT OR ETHER EXTRACT

*Direct Method—Official*

## 27.24

## REAGENT

*Anhydrous ether.*—Wash commercial ether with 2 or 3 successive portions of  $\text{H}_2\text{O}$ , add solid NaOH or KOH, and let stand until most of the  $\text{H}_2\text{O}$  has been abstracted from the ether. Decant into dry bottle, add small pieces of carefully cleaned metallic Na, and let stand until there is no further evolution of H gas. Keep ether, thus dehydrated, over metallic Na in loosely stoppered bottles.

## 27.25

## DETERMINATION

Large quantities of soluble carbohydrates may interfere with complete extraction of the fat. In such cases extract with  $\text{H}_2\text{O}$  before proceeding with the determination. Extract ca 2 g of sample, dried as directed under 27.3 or 27.7, with the anhydrous ether for 16 hours. Dry extract at temp. of boiling  $\text{H}_2\text{O}$  for 30 min., cool in desiccator, and weigh; continue, at 30 min. intervals, this alternate drying and weighing until weight is constant. For most feeds 1–1.5 hours is required.

## 27.26

*Indirect Method—Official*

Determine moisture as directed under 27.3 or 27.7; then extract dried substance for 16 hours as directed under 27.25, and dry again. Report loss in weight as ether extract.

## FAT IN DRIED MILK PRODUCTS

## 27.27

*Modified Roese-Gottlieb Method (8)—Official*

Proceed as directed in 22.102(b) and 22.103, using 8.5 ml of  $\text{H}_2\text{O}$  and 1.5 ml of  $\text{NH}_4\text{OH}$ .

## CRUDE FIBER (9)—OFFICIAL

## 27.28

## REAGENTS

(a) *Sulfuric acid soln.*—Contains 1.25 g of  $\text{H}_2\text{SO}_4$ /100 ml.

(b) *Sodium hydroxide soln.*—Contains 1.25 g of NaOH/100 ml, free, or nearly so, from  $\text{Na}_2\text{CO}_3$ .

The concn of these solns must be accurately checked by titration.

(c) *Asbestos.*—Digest on steam bath or at equivalent temp. for at least 8 hours with ca 5% NaOH soln and thoroly wash with hot  $\text{H}_2\text{O}$ ; then digest in similar man-

ner for 8 hours with HCl (1+3) and again wash thoroly with hot  $\text{H}_2\text{O}$ . Dry, and ignite at bright red heat.

## 27.29

## APPARATUS

(a) *Condenser*.—Use condenser that will maintain constant volume of soln thru-out process of digestion.

(b) *Digestion flasks*.—Use digestion flasks of such size and shape that soln will be not less than 1" nor more than 1.5" in depth. A 700–750 ml Erlenmeyer flask is recommended.

(c) *Filtering cloth*.—Use filtering cloth of such character that no appreciable solid matter passes thru when filtering is rapid. Butcher's line or dress linen with ca 45 threads to the inch or No. 40 filtering cloth made by National Filter Cloth and Weaving Company, 1717 Dixwell Ave., Hamden, Conn or its equivalent, may be used.

## 27.30

## DETERMINATION

Extract 2 g of dry material with ordinary ether, use residue from ether extract determination (27.25 or 27.26), and transfer residue, together with ca 0.5 g of asbestos, to digestion flask. (If residue from the ether extract is used and proper quantity of asbestos has already been added, further addition is unnecessary.) Add 200 ml of the boiling  $\text{H}_2\text{SO}_4$  soln, immediately connect digestion flask with condenser, and heat. (It is essential that contents of flask come to boiling within 1 min. and that the boiling continue briskly for exactly 30 min.) Rotate flask ca every 5 min. in order to mix charge thoroly. Take care to keep material from remaining on sides of flask out of contact with soln. (Blast of air conducted into flask will serve to reduce frothing of liquid.) At expiration of 30 min. remove flask, immediately filter thru linen in fluted funnel, and wash with boiling  $\text{H}_2\text{O}$  until washings are no longer acid. Bring a quantity of the NaOH soln to boiling and keep at this temp. under reflux condenser until used. Wash charge and asbestos back into flask with 200 ml of the boiling NaOH soln, using wash bottle marked to deliver 200 ml. (The boiling NaOH soln is conveniently transferred to the wash bottle by means of bent tube thru which liquid is forced by blowing into tube connected with top of the reflux condenser attached to the NaOH flask.) Connect flask with reflux condenser and boil for exactly 30 min., so timing the boiling with the alkali that contents of different flasks will reach boiling point ca 3 min. apart, which permits sufficient time for filtration. At expiration of 30 min. remove flask and immediately filter thru Gooch prepared with asbestos mat, thru alundum crucible, or thru the filtering cloth in fluted funnel. If the filtering cloth is used, thoroly wash residue with boiling  $\text{H}_2\text{O}$  and transfer it to Gooch crucible prepared with thin but close layer of ignited asbestos. After thoro washing with boiling  $\text{H}_2\text{O}$ , wash with ca 15 ml of alcohol. Dry crucible and contents at  $110^\circ$  to constant weight. Cool in efficient desiccator and weigh. Incinerate contents of crucible in electric muffle or over Meker burner at dull red heat until carbonaceous matter has been consumed (ca 20 min.). Cool in desiccator and weigh. Report loss in weight as crude fiber.

## 27.31

## REDUCING SUGARS (10)—OFFICIAL

Place 10 g of the material in 250 ml volumetric flask. If substance has acid reaction, add 1–3 g of  $\text{CaCO}_3$  to neutralize acidity. Add 125 ml of 50% alcohol by volume, mix thoroly, and boil on steam bath for 1 hour, using small funnel in neck of flask to condense vapor. Cool, and allow mixture to stand several hours, preferably overnight. Make up to volume with neutral 95% alcohol, mix thoroly, and

allow to settle. Pipet 200 ml of supernatant soln into beaker and evaporate on steam bath to volume of 20–30 ml. Do not evaporate to dryness. A little alcohol in residue does no harm. Transfer to 100 ml volumetric flask and rinse beaker thoroly with  $H_2O$ , adding rinsings to contents of flask. Add enough saturated neutral Pb acetate soln (ca 2 ml) to produce flocculent precipitate, shake thoroly, and allow to stand 15 min. Dilute to mark with  $H_2O$ , mix thoroly, and filter thru dry filter. Add sufficient anhydrous  $Na_2CO_3$  or K oxalate to filtrate to precipitate all the Pb, again filter thru dry paper, and test filtrate with a little anhydrous  $Na_2CO_3$  or K oxalate to make sure that all the Pb has been removed.

Proceed as directed under 34.39 or 34.53, using 25 ml aliquot (representing 2 g of sample). Express results as dextrose or invert sugar.

## 27.32

## SUCROSE—OFFICIAL

Introduce 50 ml of the prepared soln, 27.31, into 100 ml volumetric flask, add piece of litmus paper, neutralize with HCl, add 5 ml of HCl, and allow inversion to proceed at room temp. as directed under 34.24(c). When inversion is complete, transfer soln to beaker, neutralize with  $Na_2CO_3$ , return soln to the 100 ml flask, dilute to mark with  $H_2O$ , filter if necessary, and determine reducing sugars in 50 ml of the soln (representing 2 g of the sample) as directed under 27.31. Calculate results as invert sugar. Subtract percentage of reducing sugars before inversion from percentage of total sugar after inversion, both calculated as invert sugar, and multiply difference by 0.95 to obtain percentage of sucrose present.

Because the insoluble material of grain or cattle food occupies some space in the flask as originally made up, it is necessary to correct for this volume. To obtain the true quantity of sugars present multiply all results by factor 0.97, as results of a large number of determinations on various materials have shown the average volume of 10 g of material to be 7.5 ml.

## STARCH

(Intended only for such materials as raw starch, potatoes, etc., including as starch the pentosans and other carbohydrate bodies that undergo hydrolysis and are converted into reducing sugars on boiling with HCl.)

## 27.33

## I. Direct Acid Hydrolysis—Official

Stir weighed quantity of sample, representing 2.5–3 g of the dry material, in beaker with 50 ml of cold  $H_2O$  for 1 hour. Transfer to filter and wash with 250 ml of cold  $H_2O$ . Heat insoluble residue 2.5 hours with 200 ml of  $H_2O$  and 20 ml of HCl (sp. gr. 1.125) in flask provided with reflux condenser. Cool, and nearly neutralize with NaOH. Complete volume to 250 ml, filter, and determine dextrose in aliquot of filtrate as directed under 34.39 or 34.53. Weight of dextrose obtained  $\times 0.90$  = weight of starch (11).

## II. Diastase Method with Subsequent Acid Hydrolysis—Official

## 27.34

## REAGENT

**Malt extract.**—Use clean, new barley malt of known efficacy and grind only as needed. Grind well, but not so fine that filtration will be greatly retarded. Prepare an infusion of the freshly ground malt just before it is to be used. For every 80 ml of the malt extract required digest 5 g of the ground malt with 100 ml of  $H_2O$ , at room temp., for 2 hours, or for 20 min. if mixture can be stirred by an electric mixer. Filter to obtain a clear extract (it may be necessary to return first portions of filtrate to filter). Mix the infusion well.

## 27.35

## DETERMINATION

Extract a quantity of the substance (ground to impalpable powder and representing 4–5 g of the dry material) on close-textured filter with 5 successive portions of 10 ml of ether; wash with 150 ml of alcohol, 10% by volume, and then with a few ml of 95% alcohol. Place residue in beaker with 50 ml of  $H_2O$ , immerse beaker in boiling  $H_2O$ , and stir constantly for 15 min., or until all starch is gelatinized; cool to 55°, add 20 ml of the malt extract, and maintain at this temp. for an hour. Heat again to boiling for a few minutes, cool to 55°, add 20 ml of the malt extract, and maintain at this temp. for an hour, or until residue treated with I soln shows no blue color upon microscopic examination. Cool, make up directly to 250 ml, and filter. Place 200 ml of filtrate in flask, add 20 ml of  $HCl$  (sp. gr. 1.125), connect with reflux condenser, and heat in boiling water bath for 2.5 hours. Cool, nearly neutralize with 10%  $NaOH$  soln, finish neutralization with  $Na_2CO_3$  soln, and dilute to 500 ml. Mix soln thoroly, pour thru dry filter, and determine dextrose in aliquot as directed under 34.39 or 34.53. Conduct blank determination on same volume of the malt extract as used with sample and correct weight of dextrose accordingly. Weight of dextrose obtained  $\times 0.90$  = weight of starch.

27.36      *III. In Presence of Interfering Polysaccharides (12)—Official*

Weigh 2–6 g (charges of 4 g for linseed meal, or 3 g for dried apple pomace, have been found to be satisfactory) of the well-mixed sample, prepared to pass freely thru sieve not less than 40 mesh to the inch, using smaller charges in case of materials containing much gel-forming substance. (Weight of starch in charge must not exceed 1.5 g.) Transfer to dry 12.5–15 cm close-textured rapid filtering paper in glass funnel and extract with 5 successive portions of ether, taking for each portion more than enough to cover charge and using cover-glass to retard evaporation. After completing ether extraction, allow ether to evaporate and then extract charge with 300 ml of dilute alcohol. Concentration of alcohol may be varied somewhat to suit material under examination. For linseed meal use 35% alcohol (by volume) and for dried apple pomace use 25% alcohol. Follow this with several filterfuls of 95% alcohol and finish leaching operations with a second ether extraction. Conduct also control determination, preferably in duplicate, using filter paper extracted with alcohol and same quantity of  $H_2O$  and malt extract as in determination. (It is convenient to let charge stand overnight at this point to allow ether and alcohol to evaporate, as alcohol must be eliminated before starting digestion with malt; or charge may be dried at ca 75° until alcohol has been eliminated.)

Transfer as much of dry material as possible from filter paper into glass mortar and pulverize all lumps. Transfer both filter paper and sample to 500 ml volumetric flask, add 20–30 ml of  $H_2O$ , and thoroly wet material by vigorous shaking.

Should more cold  $H_2O$  be needed to make material more fluid, calculate quantity of hot  $H_2O$  to be added accordingly, so that total volume allowing for 40 ml of malt soln will not exceed 200 ml. Let stand a few minutes, add 100 ml of actively boiling  $H_2O$ , and thoroly gelatinize in boiling water bath.

Cool to 50° or lower, add 20 ml of malt extract, 27.34, to controls as well as to charges, and place flasks in temp.-controlled water bath. Keeping mash thoroly mixed, gradually raise temp. to 70° in 20–30 min. Maintain at 70° for 30 min., stirring mixture from time to time, then increase temp. to 80°, and keep it at that temp. for 10 min. Finally heat to boiling point. Keep mixtures well stirred. Cool contents of flasks and water bath to 55°. Add 20 ml of the malt extract, mix well, and hold at 55° for 1 hour, stirring about once every 10 min. At termination of digestion rapidly increase temp. to above 80°.



Measure out 316 ml of alcohol. Add a portion, a little at a time, to contents of flask, with thoro shaking between additions. After cooling to room temp. adjust volume with H<sub>2</sub>O so that quantity of liquid is 500 ml, making allowance for volume occupied by charge by adding 3 ml of H<sub>2</sub>O for every 4 g of charge present after bringing contents to 500 ml mark. (Determination may be interrupted at this stage for several days, but volume should be readjusted if evaporation has occurred in meantime.) Mix thoroly, breaking up any ropy coagulum as much as possible by pouring back and forth from one large beaker to another. Filter thru dry paper. Test solid residue for starch, either microscopically or by the I color test, after elimination of alcohol and gelatinization with H<sub>2</sub>O. (If more than merest trace of starch is found, reject entire determination.) Evaporate exactly 200 ml of filtrate on steam bath to volume of 15–20 ml, or until practically all alcohol has been expelled. Do not allow evaporation to proceed to dryness.

Transfer aqueous residue of starch conversion products to 200 ml volumetric flask with hot H<sub>2</sub>O, using policeman to recover any dextrin that may be present. Allow to cool somewhat, and complete volume to 200 ml. Transfer contents to suitable digestion flask, add 20 ml of HCl (sp. gr. 1.125), made by diluting 68 ml of strong acid (sp. gr. 1.19, or 37% HCl) to 100 ml, and connect flask with reflux condenser. Heat in boiling water bath for 2.5 hours. Cool, and for samples of linseed meal or other material yielding solns which at this stage need further purification, add not more than 1 ml of 10% soln of phosphotungstic acid in 1% HCl. Mix, and allow to stand at least 15 min. Increase volume with H<sub>2</sub>O to 250 ml in volumetric flask, mix well, and filter thru dry paper. Partially neutralize 200 ml of filtrate while stirring by adding 10 ml of conc. NaOH soln (44 g of NaOH/100 ml of H<sub>2</sub>O) and nearly complete neutralization with a little powdered anhydrous Na<sub>2</sub>CO<sub>3</sub>. Transfer to 250 ml flask with H<sub>2</sub>O, cool to room temp., make up to mark, and thoroly mix. Filter, if necessary, and determine dextrose in 50 ml aliquot of the filtrate, gravimetrically, as directed under 34.39 or 34.53. Correct weight of dextrose obtained by subtracting weight of dextrose found for same aliquot of the malt control, and multiply corrected weight of dextrose by 0.90 to obtain weight of starch.

$$\text{Aliquots: Charge} \times \frac{200}{500} \times \frac{200}{250} \times \frac{50}{250}, \quad \text{or} \quad \text{Charge} \times 0.064.$$

#### 27.37 IV. In Condensed or Dried Milk Products—Qualitative Test (13)—Official

Mix ca 2 g of sample with 100 ml of H<sub>2</sub>O and boil mixture 2 min. Place a few ml of cooled mixture on spot plate or in test tube and add a drop of I-KI test soln (0.05 g of I and 0.2 g of KI dissolved in 15 ml of H<sub>2</sub>O). If starch is present, blue color will be produced.

#### PENTOSANS (14)—OFFICIAL

##### 27.38

##### REAGENTS

(a) *Hydrochloric acid*.—Contains 12% by weight of HCl. To 1 volume of HCl add 2 volumes of H<sub>2</sub>O. Determine percentage of acid by titration against standard alkali and adjust to proper concn by dilution or addition of more HCl, as may be necessary.

(b) *Phloroglucin*.—Dissolve small quantity of phloroglucin in a few drops of acetic anhydride, heat almost to boiling, and add a few drops of H<sub>2</sub>SO<sub>4</sub>. Violet color indicates presence of diresorcin. A phloroglucin that gives more than a faint coloration may be purified by following method: Heat in beaker ca 300 ml of the dilute HCl and 11 g of commercial phloroglucin, added in small quantities at a time, stirring constantly until it is nearly dissolved. Pour hot soln into sufficient quantity of same HCl (cold) to make volume 1500 ml. Allow to stand at least overnight, preferably

several days, to permit diorescin to crystallize. Filter immediately before using. Yellow tint does not interfere with its usefulness. In using, add volume containing required quantity of phloroglucin to distillate.

## 27.39

## DETERMINATION

Place in 300 ml distillation flask such a quantity of sample, 2-5 g, that weight of phloroglucide obtained will not exceed 0.300 g, together with 100 ml of the dilute HCl and several pieces of recently ignited pumice stone. Place flask on wire gauze, connect with condenser, and heat, rather gently at first, and then regulating so as to distil over 30 ml in ca 10 min. Pass distillate thru small filter paper. Replace the 30 ml distilled by like quantity of the dilute acid, added by means of separator in such manner as to wash down particles adhering to sides of flask, and continue process until distillate amounts to 360 ml. To total distillate add gradually a quantity of phloroglucin dissolved in the dilute HCl and thoroly stir resulting mixture. (Quantity of phloroglucin used should be about double that of furfural expected. Soln turns yellow, then green, and soon there appears an amorphous greenish precipitate that grows darker rapidly, till it becomes almost black.) Make soln to 400 ml with the dilute HCl and allow to stand overnight.

Collect the amorphous black precipitate in weighed Gooch crucible having an asbestos mat, wash carefully with 150 ml of H<sub>2</sub>O so that the H<sub>2</sub>O is not entirely removed from crucible until the very last, and dry 4 hours at temp. of boiling H<sub>2</sub>O. Cool, and weigh in weighing bottle. Increase in weight is considered to be furfural phloroglucide. To calculate furfural, pentoses, or pentosans from phloroglucide, use following formulas given by Kröber:

(1) For a weight of phloroglucide, designated by "a" in following formulas, *under* 0.03 g:

$$\text{Furfural} = (a + 0.0052) \times 0.5170.$$

$$\text{Pentoses} = (a + 0.0052) \times 1.0170.$$

$$\text{Pentosans} = (a + 0.0052) \times 0.8949.$$

In preceding and also in following formulas, the factor 0.0052 represents weight of the phloroglucide that remains dissolved in the 400 ml of acid soln.

(2) For a weight of phloroglucide "a" *between* 0.03 and 0.300 g, use Kröber's table, 44.22, or the following formulas (15):

$$\text{Furfural} = (a + 0.0052) \times 0.5185.$$

$$\text{Pentoses} = (a + 0.0052) \times 1.0075.$$

$$\text{Pentosans} = (a + 0.0052) \times 0.8866.$$

(3) For a weight of phloroglucide "a" *over* 0.300 g, use following formulas:

$$\text{Furfural} = (a + 0.0052) \times 0.5180.$$

$$\text{Pentoses} = (a + 0.0052) \times 1.0026.$$

$$\text{Pentosans} = (a + 0.0052) \times 0.8824.$$

## 27.40

## GALACTAN—TENTATIVE

Extract convenient quantity of sample, representing 2.5-3 g of dry material, on hardened filter with 5 successive portions of 10 ml of ether; place extracted residue in beaker, ca 5.5 cm in diam. and 7 cm deep; add 60 ml of HNO<sub>3</sub> (sp. gr. 1.15); and evaporate on steam bath to volume of 20 ml. Let stand 24 hours, add 10 ml of H<sub>2</sub>O, and allow to stand another 24 hours. Pass mixture thru filter, wash impure mucic acid crystals with 30 ml of H<sub>2</sub>O to remove as much of the HNO<sub>3</sub> as possible, and return filter and contents to original beaker. Add 30 ml of (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub> soln (con-

sisting of 1 part "ammonium carbonate," 19 parts  $H_2O$ , and 1 part of  $NH_4OH$ ) and heat mixture in water bath, at  $80^\circ$ , for 15 min., with constant stirring. The  $(NH_4)_2CO_3$  combines with mucic acid, forming soluble  $NH_4$  mucate. Wash filter paper and contents several times with hot  $H_2O$  by decantation, passing washings thru filter paper, to which finally transfer residue and wash thoroly. Evaporate filtrate to dryness on water bath, avoiding unnecessary heating, which causes decomposition; add 5 ml of  $HNO_3$  (sp. gr. 1.15); stir mixture thoroly; and allow to stand 30 min. Collect precipitated mucic acid on weighed Gooch crucible or other filter; wash with 10-15 ml of  $H_2O$ , then with 60 ml of alcohol, and then a number of times with ether; dry at temp. of boiling  $H_2O$  for 3 hours; and weigh. Multiply weight of mucic acid by 1.33 to convert to galactose, and by 1.20 to convert to galactan.

**27.41 WATER-SOLUBLE ACIDITY (16)—OFFICIAL, FIRST ACTION**

Weigh 10 g of sample into shaking bottle, add 200 ml of  $H_2O$ , and shake for 15 min. Filter extract thru folded filter and take 20 ml aliquot (equivalent to 1 g of sample). Dilute with 50 ml of  $H_2O$  and titrate with 0.1 *N* NaOH, using phenolphthalein indicator. Report results in terms of ml of 0.1 *N* NaOH required for neutralizing extract from 1 g of material.

**27.42 SALT (17) (QUALITATIVE)—OFFICIAL**

Transfer 2 ml of 5%  $AgNO_3$  soln to small test tube of 1 cm internal diam. Carefully add to this liquid an equal volume of the feed, which previously has been ground to pass 1 mm sieve, so that most of sample floats or remains above liquid. Gradually incline tube so that liquid is absorbed. White patches of  $AgCl$  appear wherever minutest crystal of  $NaCl$  comes in contact with soln. These patches may easily be observed with a lens or even with naked eye.

**27.43 RICE HULLS IN RICE BRAN (18)—OFFICIAL, FIRST ACTION**

Thoroly mix sample to be examined. Withdraw small portion and grind until it passes thru 60-mesh sieve. Weigh 4 mg on slide ruled in parallel lines  $1/20''$  apart or transfer to ruled slide after weighing. Add just sufficient chloral hydrate soln (1+1) to fill in under cover-glass, which, preferably, should be square (ca 22 mm). After cover-glass is in place, warm gently, but do not boil, to eliminate starch masses and clear tissues. Count particles of hull tissue, using microscope having magnification of ca 90 diameters. The high refraction and yellowish green color of hull particles will aid in distinguishing the small pieces not easily recognized by their structure. (In order to avoid duplicate counting, it is well to disregard the particles that extend over upper line of strip.) Compare results with those obtained on standards containing known quantities of hulls.

**27.44 OAT HULLS IN OATS AND OAT FEEDS (19)—OFFICIAL, FIRST ACTION**

(Results are only approximate)

Place in 1000 ml beaker 800 ml of  $H_2O$  and 2 g of sample, previously ground to pass thru sieve having circular openings 1 mm in diam. Stir vigorously to obtain a centrifugal effect, allow to stand 5 min., and decant supernatant liquid carefully, retaining so far as possible all hull particles. Repeat procedure several times until supernatant liquid becomes clear, or nearly so, and then transfer residue with aid of 150 ml of  $H_2O$  to 300 ml beaker. Add 5 drops of  $HCl$  and boil 2 min., constantly stirring mixture. Transfer to original beaker with aid of 500 ml of  $H_2O$ , stir, and allow to stand until supernatant liquid is clear. Draw off liquid by means of siphon

of rubber tubing having 3 or 4 mm bore, using pinch clamp to control flow so that practically all liquid may be siphoned off. (Tilting beaker will also help to obtain this result.) If on standing a deposit forms, siphon again. Transfer hulls with aid of  $H_2O$  to paper filter, wash several times with alcohol, and allow to dry to constant weight at room temp. When dry, carefully remove hulls from paper, using if necessary small stiff brush, and weigh. (Weighed Gooch crucible may be used instead of paper filter.) Multiply weight of hulls by 50 to obtain percentage of hulls in sample.

**27.45****GRIT IN POULTRY AND SIMILAR FEEDS (#0)—OFFICIAL**

Place 2 g of prepared sample, 27.2, thoroly mixed, in evaporating dish of ca 30 ml capacity. Add ca 5 ml of  $CHCl_3$  and mix gently with glass rod until liquid comes in contact with all portions of sample. Brush particles adhering to rod into dish, and after pushing all particles down into the  $CHCl_3$  with 25 mm circular or square cover-glass, use glass to skim off or pull floating portion of material over top of dish, taking care not to submerge cover-glass deep enough to disturb grit settled at bottom of dish. After skimming until surface of the  $CHCl_3$  is nearly clear, slowly pour supernatant liquid into second evaporating dish. Wash sides of dish with a few ml more of  $CHCl_3$  and repeat skimming and decanting operation until no floating particles remain (10–15 ml of  $CHCl_3$ ). When only grit remains, allow last traces of  $CHCl_3$  to evaporate spontaneously, and weigh. Weight of residue  $\times 50$  = percentage of grit. After weighing examine residue for impurities. Also pour out  $CHCl_3$  washings collected in second dish and observe whether any grit has been transferred to it during process.

If sample contains NaCl, remove from grit by washing with  $H_2O$ . Identify bone in grit by charring. If pelleted feeds or feeds containing molasses are being examined, disintegrate in cold  $H_2O$  and dry with alcohol or ether.

**27.46****BONE IN MEAT SCRAP OR TANKAGE (#1)—OFFICIAL**

Separate bone as directed under 27.45. In some instances it may be found necessary, after first washing with  $CHCl_3$ , to rub remaining residue of bone with glass rod or small pestle in order to bring some of adhering particles to surface of the  $CHCl_3$ .

**27.47****CALCIUM OXIDE IN MINERAL FEEDS (#2)—OFFICIAL**

Weigh 2 g portion of finely ground sample into  $SiO_2$  or porcelain dish and ignite in muffle to C-free ash, but avoid fusing. Boil residue in 40 ml of HCl (1+3) and a few drops of  $HNO_3$ . Transfer to 250 ml volumetric flask, cool, dilute to mark, and mix thoroly. Pipet 25 ml of clear liquid into beaker, dilute to ca 100 ml, and add 2 drops of methyl red indicator. Add  $NH_4OH$  (1+1) dropwise to pH of 5.6, as shown by intermediate brownish orange color. If over-stepped, add with dropper HCl (1+3) to orange point. Add 2 drops HCl (1+3). The color should now be pink (pH 2.5–3.0) instead of orange. Dilute to ca 150 ml, bring to boiling, and add slowly with constant stirring 10 ml of saturated (4.2%) soln of  $(NH_4)_2C_2O_4$ , which should also be hot. If red color changes to orange or yellow, add HCl (1+3) dropwise until color again changes to pink. Let stand overnight to allow precipitate to settle. Filter supernatant liquid thru quantitative filter paper on Gooch crucible, or on fritted glass filter (Jena 1G4 is preferable), and wash precipitate thoroly with  $NH_4OH$  (1+50). Place filter paper or crucible with precipitate in original beaker, and add mixture of 125 ml of  $H_2O$  and 5 ml of  $H_2SO_4$ . Heat to 70° or above and titrate with 0.1 N  $KMnO_4$  until first slightly pink color is obtained. Presence of filter paper may cause pink color to fade in few seconds. Correct for blank and calculate percentage of CaO in sample.

## CYANOGENETIC GLUCOSIDES IN FEEDS AND SIMILAR MATERIALS (23)

27.48

*Qualitative Test—Official*

Prepare Na picrate paper by dipping strips into 1% picric acid soln and drying, then dipping into 10%  $\text{Na}_2\text{CO}_3$  soln and drying. Preserve these papers in stoppered bottle. Finely chop small quantity of plant material and place in test tube. Insert piece of the moist Na picrate paper in tube, taking care that it does not come in contact with material. Add a few drops of  $\text{CHCl}_3$  and stopper tube tightly. The Na picrate paper gradually turns orange, then brick red, if plant tissue contains cyanogenetic glucosides. (Test is delicate, and rapidity of change in color depends upon amount of free HCN present. This test works well with fresh plant materials, but in the case of relatively dry substances, particularly seeds of various plants, material should be ground and moistened with  $\text{H}_2\text{O}$  and allowed to hydrolyze in stoppered test tube containing Na picrate paper. If necessary, small amount of emulsin may be added.)

## HYDROCYANIC ACID FORMED BY HYDROLYSIS OF GLUCOSIDES IN BEANS (24)

27.49

*Acid Titration Method—Tentative*

Introduce 10–20 g of sample, ground to pass 20-mesh sieve, into 800 ml Kjeldahl<sup>1</sup> flask, add 100 ml of  $\text{H}_2\text{O}$ , and macerate at room temp. for 2 hours. Add 100 ml of  $\text{H}_2\text{O}$  and distil with steam, collecting distillate in 20 ml of 0.02  $N$   $\text{AgNO}_3$  acidified with 1 ml of  $\text{HNO}_3$ . Before distilling, adjust apparatus so that tip of condenser dips below surface of liquid in receiver. When 150 ml has passed over, filter distillate thru Gooch crucible; wash receiver and Gooch with a little  $\text{H}_2\text{O}$ ; and titrate excess of  $\text{AgNO}_3$  in combined filtrate and washings with 0.02  $N$  KCNS, using Fe alum indicator. 1 ml of 0.02  $N$   $\text{AgNO}_3$  = 0.54 mg. of HCN.

27.50

*Alkaline Titration Method—Tentative*

Place 10–20 g of sample, ground to pass 20-mesh sieve, in 800 ml Kjeldahl flask, add ca 200 ml of  $\text{H}_2\text{O}$ , and allow to stand 2–4 hours. (Autolysis should be conducted with apparatus completely connected for distillation.) Distill with steam and collect 150–160 ml distillate in soln of NaOH (0.5 g in 20 ml of  $\text{H}_2\text{O}$ ).

To 100 ml of distillate (it is preferable to dilute to volume of 250 ml and titrate 100 ml aliquot) add 8 ml of 6  $N$   $\text{NH}_4\text{OH}$  and 2 ml of 5% KI soln and titrate with 0.02  $N$   $\text{AgNO}_3$ , using microburet. End point is faint but permanent turbidity and may be easily recognized, especially against black background. 1 ml of 0.02  $N$   $\text{AgNO}_3$  = 1.08 mg of HCN.

27.51

## FERROUS SULFATE (25)—OFFICIAL

Sift a portion of feed thru fine sieve (40-mesh) over sheet of white glazed paper whose entire surface has been moistened with  $\text{K}_3\text{Fe}(\text{CN})_6$  soln (1+10) in such manner that it will be distributed thinly over entire area. After few moments wash off feed under slow stream of  $\text{H}_2\text{O}$ . Blue speck or spot denotes particle of ferrous salt.

27.52

## COPPER SULFATE (26)—OFFICIAL

Proceed as directed under 27.51, except to use  $\text{K}_4\text{Fe}(\text{CN})_6$  soln (1+10). Brown speck or spot denotes particle of Cu salt.

27.53

## POTASSIUM IODIDE (25)—OFFICIAL

So sift portion of feed over sheet of white glazed paper whose entire surface has been moistened with mixture of starch indicator and Br water (3 parts of former to

1 of latter) that feed will be distributed thinly over entire area. Blue coloration denotes particle of an iodide. If an extremely small quantity of KI is to be detected, modify procedure by carefully charring 10 g or more of the feed, washing residue with small amount of  $H_2O$  and evaporating filtered soln in white evaporating dish so that solids are concentrated on one small spot. When moistened with the starch indicator and Br water, blue coloration denotes presence of an iodide.

#### IODINE IN MINERAL MIXED FEEDS

##### *Knapheide-Lamb Method (26)—Tentative*

#### 27.54

##### REAGENTS

(a) *Reduced phosphoric acid.*—20%. Reduce impurities in the  $H_3PO_4$  according to Kendall's method (27) by diluting the 85% acid with 4 volumes of  $H_2O$  and boiling for some time with Al strips.

(b) *Sodium thiosulfate soln.*—0.005 N. Preferably standardize as follows: Pipet into beaker 25 ml of soln containing 0.1308 g of KI/liter and add 200 ml of  $H_2O$ , 5 ml of 20%  $NaHSO_3$  soln, and 2 or 3 g of NaOH. Neutralize mixture with sirupy  $H_3PO_4$ , add 1 ml in excess, and proceed as directed in regular determination. To calculate mg of I to which 1 ml of the  $Na_2S_2O_3$  soln is equivalent, use following formula:

$$\frac{2.5}{\text{ml of } Na_2S_2O_3 \text{ soln}} \cdot (\text{It is well to standardize the } Na_2S_2O_3 \text{ soln same day determination is made.})$$

#### 27.55

##### APPARATUS (28)

*Furnace*—Use sheet Fe cylinder 4" in diam. and 12" high, having opening in center of top large enough to accommodate 100 ml Ni crucible. Suspend  $2\frac{1}{2}$ " circular plate in center of cylinder 3" below top, for spreading flame, thereby preventing free flame from coming in contact with crucible, and providing uniform heat. Make slot at bottom of cylinder 1" wide by 3" high for admitting air and the burner tubing, and near top rim make eight  $\frac{1}{8}$ " holes to allow for escape of exhaust gases.

#### 27.56

##### DETERMINATION

Fuse together in 100 ml Ni crucible 20 g of NaOH and 10 g of  $KNO_3$ , and cool. Place evenly on top of fused alkali 1–10 g of sample (depending upon its composition and trouble experienced from frothing in fusion) and completely moisten with 5 ml of NaOH soln (1+1) and 10 ml of 80% alcohol. Place crucible on cold three-heat hot plate and evaporate alcohol at low heat. After 30 min. cautiously increase heat until crucible has been subjected to highest temp. of hot plate for 1½–2 hours. (Thoro heating at this stage prevents most of trouble from effervescence of material during fusion.) Then place crucible in furnace described previously or in similar furnace.

To prevent loss give close attention during fusion to mineral mixtures containing charcoal or organic matter because of violent reaction between the C and the  $KNO_3$ . If reaction becomes too violent, lift crucible from furnace for a moment, and if necessary cool bottom of crucible in beaker of  $H_2O$ . When mixture is in quiet state of fusion tip crucible on all sides in open flame to wash down fusion mixture. Add a few small crystals of  $KNO_3$  until no more gas is liberated by further additions, and again wash down sides of crucible in flame.

Pour melt out into clean crucible cover to cool, or turn crucible while cooling so that material solidifies on sides. Place cooled melt and crucible in 600 ml beaker, cover with  $H_2O$ , and heat below boiling point for short time. After allowing mixture

to stand overnight at room temp., rinse off crucible and cover and remove. In order to neutralize part of alkali and facilitate filtering, add 10 ml of sirupy  $\text{H}_3\text{PO}_4$  and place beaker on steam bath for 3–4 hours, stirring occasionally to break up mass and insure complete solution of the I. Cool beaker, filter off insoluble residue in 10 cm funnel, and wash with cold  $\text{H}_2\text{O}$  into 800 ml beaker, adjusting volume to 550–600 ml. (Soln should be clear and colorless.)

In order to destroy nitrites, which interfere with titration with methyl orange, add 10 ml of 20%  $\text{NaHSO}_3$  soln, bring soln just to boiling point, and cool. Run ca 30 ml of 85%  $\text{H}_3\text{PO}_4$  in from buret, add a few drops of methyl orange soln, continue addition of  $\text{H}_3\text{PO}_4$  to neutral color of methyl orange, and finally add 1.5 ml of  $\text{H}_3\text{PO}_4$  in excess. (Total quantity of  $\text{H}_3\text{PO}_4$  required is generally not over 35 ml, except when presence of considerable C in sample has necessitated use of more  $\text{KNO}_3$ , which is mainly reduced to carbonate.) Use care not to run appreciably over end point, as excess acid gives low results. However, addition of acid must be fairly rapid, as color of methyl orange has tendency to fade, due to incomplete destruction of nitrites.

After neutralization, add small lump of anthracite coal (0.5 cm in diam.) and boil soln at least 20 min., reducing volume to 400–500 ml. (Boiling is essential to remove all traces of  $\text{SO}_2$ .) Again cool soln and add Br water until distinct and permanent yellow color is produced. Boil soln until colorless by reflected light and then for exactly 5 min. longer. Add a few crystals of salicylic acid to assure removal of last traces of Br, cool soln, and add 5 ml of 20% reduced  $\text{H}_3\text{PO}_4$  and 0.5–1.0 g of KI. Titrate soln in usual manner with 0.005 *N*  $\text{Na}_2\text{S}_2\text{O}_3$ , adding starch soln when brown color of liberated I is nearly gone. (Volume of soln at final titration should be 400–500 ml.)

## 27.57

*Elmslie-Caldwell Method (29)—Tentative*

Place a sample that contains 3–4 mg of I in 200–300 ml Ni dish. Add ca 5 g of  $\text{Na}_2\text{CO}_3$ , 5 ml of  $\text{NaOH}$  soln (1+1), and 10 ml of alcohol, taking care that entire sample is moist. Dry at ca  $100^\circ$ , so that there will be no spattering upon subsequent heating (30 min. is usually sufficient).

Place dish and contents in muffle furnace, heated to  $500^\circ$ , and maintain at that temp. 15 min. (Ignition of sample to  $500^\circ$  appears to be necessary only to carbonize any soluble organic matter that would be oxidized by Br water if not so treated. Temps. higher than  $500^\circ$  may be used if necessary.) Cool, add 25 ml of  $\text{H}_2\text{O}$ , cover dish with watch-glass, and boil gently for 10 min. Transfer contents of dish to 18 cm filter paper and wash with boiling  $\text{H}_2\text{O}$ , catching filtrate and washings in 600 ml beaker (soln should total ca 300 ml). Neutralize to methyl orange with 85%  $\text{H}_3\text{PO}_4$  and add 1 ml in excess.

Add excess of Br water and boil soln gently until colorless, and then 5 min. longer. Add a few crystals of salicylic acid and cool soln to ca  $20^\circ$ . Add 1 ml of 85%  $\text{H}_3\text{PO}_4$  and ca 0.5 g of KI and titrate I with 0.005 *N*  $\text{Na}_2\text{S}_2\text{O}_3$  as directed under 27.54(b).

## ACID-SOLUBLE MANGANESE (30)—OFFICIAL

## 27.58

## REAGENT

*Standard potassium permanganate soln.*—Dissolve 1.4385 g of  $\text{KMnO}_4$  by boiling with  $\text{H}_2\text{O}$ . Dilute to 1 liter, let stand several days, and filter thru asbestos pad on Gooch crucible. Standardize with Na oxalate (soln should contain 500 p.p.m. of Mn). Transfer aliquot containing 20 mg of Mn to beaker. Add 100 ml of  $\text{H}_2\text{O}$ , 15 ml of  $\text{H}_3\text{PO}_4$ , and 0.3 g of  $\text{KIO}_4$ , and bring to b.p. Cool, and dilute to 1 liter. Protect from light. Dilute this soln containing 20 p.p.m. of Mn with  $\text{H}_2\text{O}$  (that has been boiled

with 0.3 g of  $\text{KIO}_4$  per liter) to make convenient working standards of known concentrations approximately like those to be compared.

## 27.59

## DETERMINATION

Ash weighed sample, 5–15 g, at dull red heat in porcelain dish. When cool, add 5 ml of  $\text{H}_2\text{SO}_4$  and 5 ml of  $\text{HNO}_3$  to ash in dish or to ash transferred to beaker with 20–30 ml of  $\text{H}_2\text{O}$ . Evaporate to white fumes. If C is not completely destroyed, add further portions of  $\text{HNO}_3$ , boiling after each addition. Cool slightly, transfer to 50 or 100 ml volumetric flask, and add a volume of  $\text{H}_3\text{PO}_4$  soln (8 ml of  $\text{H}_3\text{PO}_4$  + 92 ml of  $\text{H}_2\text{O}$ ) equal to  $\frac{1}{2}$  volume of flask (25–50 ml). Cool, make to volume, mix, and filter or let stand until clear. If 50 ml flask was used, pipet 25 ml of clear soln into beaker or 50 or 100 ml volumetric flask and add 15 ml of  $\text{H}_2\text{O}$ . If 100 ml flask was used, pipet 50 ml into beaker or 100 ml flask and add 30 ml of  $\text{H}_2\text{O}$ . Heat nearly to boiling point, and with stirring or swirling add 0.3 g of  $\text{KIO}_4$  for each mg of Mn present. Compare with the standard  $\text{KMnO}_4$  soln in colorimeter. Calculate p.p.m. of Mn in sample.

## SOLUBLE CHLORINE (31)—OFFICIAL

## 27.60

## REAGENTS

(a) *Potassium chloride soln.*—Recrystallize reagent KCl three times from  $\text{H}_2\text{O}$ , dry at  $110^\circ$ , then heat at ca  $500^\circ$  to constant weight. Dissolve 2.1026 g in distilled  $\text{H}_2\text{O}$  and dilute to 1 liter. Soln contains 0.001 g of Cl/ml.

(b) *Silver nitrate soln.*—Dissolve 5 g of  $\text{AgNO}_3$  in 1 liter of  $\text{H}_2\text{O}$  and adjust soln so that 1 ml = 1 ml of standard KCl soln.

(c) *Potassium thiocyanate soln.*—Dissolve 2.5 g of KSCN in 1 liter of  $\text{H}_2\text{O}$  and adjust so that 1 ml = 1 ml of standard  $\text{AgNO}_3$  soln. Standardize as directed in 43. 26(d).

(d) *Ferric sulfate soln.*—Dissolve 60 g of  $\text{Fe}_2(\text{SO}_4)_3$  + Aq in distilled  $\text{H}_2\text{O}$  and dilute to 1 liter.

(e) *Ferric sulfate indicator.*—To 25% filtered soln of  $\text{Fe}_2(\text{SO}_4)_3$  + Aq add an equal volume of  $\text{HNO}_3$ .

## 27.61

## DETERMINATION

Transfer 3 g to 300 ml Erlenmeyer flask. Add 50 ml of the  $\text{Fe}_2(\text{SO}_4)_3$  soln (accurately measured with pipet or other accurately calibrated dispensing apparatus). Swirl flask during addition of the  $\text{Fe}_2(\text{SO}_4)_3$  soln to prevent caking of sample and to facilitate soln of Cl. Add 100 ml (also accurately measured) of  $\text{NH}_4\text{OH}$  (1+19). Swirl flask enough to insure soln of Cl and thoro mixing of soln. (Very little swirling is necessary. If soln is agitated by vigorous vertical shaking, difficulty will be experienced in filtering.) Allow mixture to settle 10 min. Filter thru dry No. 41 Whatman 11 cm filter paper or thru filter paper of ca same speed and retentiveness. Use 50 ml aliquots (representing one-third of total) on samples low in Cl (0–2% Cl), and 25 ml aliquots (representing one-sixth of total) on samples high in Cl (over 2%). For mineral and other feeds containing over 10% Cl, weigh 1 g and use 15 ml (representing one-tenth of total).

If approximate percentage of Cl in sample is not known, it may be well to take 10 ml aliquot for trial titration. To this add 10 ml of  $\text{HNO}_3$  + 10 ml of the  $\text{Fe}_2(\text{SO}_4)_3$  indicator. Dilute to ca 50 ml. Add 0.5 ml of the KSCN soln and immediately add with stirring enough  $\text{AgNO}_3$  soln to entirely eliminate any reddish color. From this titration calculate volume of  $\text{AgNO}_3$  soln necessary to precipitate all Cl in aliquot to be used, adding excess equal to ca 10% of total volume necessary, although somewhat greater excess will not affect results. Minimum total of 10 ml should be used.



To sample aliquot in 250 ml beaker, add 10 ml of  $\text{HNO}_3$  and 10 ml of the  $\text{Fe}_2(\text{SO}_4)_3$  indicator (or 20 ml of soln containing equal volumes of these solns). Then add, with stirring, calculated volume of  $\text{AgNO}_3$ . Heat to boiling and allow to cool to room temp., stirring enough to coagulate precipitate. (Cooling may be hastened by immersion of beakers in cold  $\text{H}_2\text{O}$ .) Titrate excess of  $\text{AgNO}_3$  with  $\text{KSCN}$ . End point is indicated by first appearance of reddish tint that persists for 15 seconds. For accurate work use a reference soln containing all ingredients except  $\text{KSCN}$ . End point is first change in color.

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## 28. MEAT AND MEAT PRODUCTS

### MEAT

#### 28.1

##### PREPARATION OF SAMPLE—OFFICIAL

To prevent loss of  $H_2O$  during preparation and subsequent handling do not use small samples. Keep the ground material in glass or similar containers provided with air- and water-tight covers. Prepare samples for analysis in following manner:

(a) *Fresh meats, dried meats, cured meats, smoked meats, etc.*—Separate as completely as possible from any bone; pass rapidly thru food chopper 3 times, thoroly mixing after each grinding; and begin all determinations as soon as practicable. If any delay occurs, chill sample to inhibit decomposition.

(b) *Canned meats.*—Pass entire contents of can thru food chopper, as directed under (a).

(c) *Sausages.*—Remove from casings and pass thru food chopper, as directed under (a).

Dry the portions of the samples under (a), (b), and (c) not needed for immediate analysis, either in vacuo below  $60^\circ$  or by evaporating on steam bath 2 or 3 times with alcohol. Extract fat from dried product with gasoline (b.p. below  $60^\circ$ ) and allow gasoline to evaporate spontaneously, finally expelling last traces by heating for short time on steam bath. Do not heat sample or separated fat longer than necessary because of tendency to decompose. Reserve fat for examination as directed under Chap. 31, keeping it in cool place, and complete examination before it becomes rancid.

#### 28.2

##### MOISTURE—OFFICIAL

Proceed as directed under 27.3 or 27.7.

#### 28.3

##### ADDED WATER IN SAUSAGE (1)—TENTATIVE

(a) *Moisture.*—Weigh accurately ca 10 g of the ground sample into tared weighing bottle ca 2" in diam., containing short glass rod flattened at one end. Remove 2.5–3 g for protein determination. Reweigh remainder in bottle, spreading it out in thin layer over sides and bottom by means of the glass rod, and use this sample for determination of moisture. Dry in air at atmospheric pressure at temp. of  $101$ – $102^\circ$  16–18 hours, or at temp. of ca  $125^\circ$  (not lower than  $120^\circ$  nor higher than  $130^\circ$ ) 2–3 hours, or until no significant loss of weight occurs on subsequent drying for period of 1–2 hours. If preferred, determine moisture as directed under 27.3 or 27.7.

(b) *Nitrogen.*—Determine total N as directed under 2.24, 2.25, or 2.26. Protein = total N  $\times 6.25$ .

(c) *Added water.*—Multiply percentage of protein calculated from N determination (b) by 4 and subtract result from percentage of moisture found. Report difference, if any, as added  $H_2O$ .

#### 28.4

ASH—OFFICIAL.—See 34.9 or 34.10

#### 28.5

##### SALT (2)—OFFICIAL

Moisten 2½–3 g of the finely comminuted and thoroly mixed sample in Pt dish with 20 ml of 5%  $Na_2CO_3$  soln, evaporate to dryness, and ignite at temp. not exceeding dull redness. Extract with hot  $H_2O$ , filter, and wash. Return residue to dish

and ignite to an ash. Dissolve ash in  $\text{HNO}_3$  (1+4), filter to free from any insoluble residue, wash thoroly, and add wash soln to the  $\text{H}_2\text{O}$  extract. Determine Cl in combined filtrate and washings as directed in 12.44.

#### 28.6 CRUDE FAT OR ETHER EXTRACT—OFFICIAL

Dry 3–4 g of sample as directed under 28.3. Grind dried sample with asbestos, sand, or similar substance, and proceed as directed under 27.25.

#### 28.7 TOTAL PHOSPHORUS—OFFICIAL

Destroy organic matter as directed under 2.8(c) or (d), and proceed as directed under 2.9 or 2.12.

#### 28.8 TOTAL NITROGEN (S)—OFFICIAL

Proceed as directed under 2.24, 2.25, or 2.26, using ca 2 g of the fresh sample.

#### AMMONIA

##### *Aeration Method (4)—Tentative*

#### 28.9 APPARATUS

Use apparatus illustrated in Fig. 41. *A* is wash bottle  $\frac{1}{2}$  full of  $\text{H}_2\text{SO}_4$  (1+9); *B* is tube containing sample; *C* is rubber disk; and *D* is 5 ml bulb to prevent spray from being carried over into tube *E*, which contains the standard acid; *F* is safety bottle.

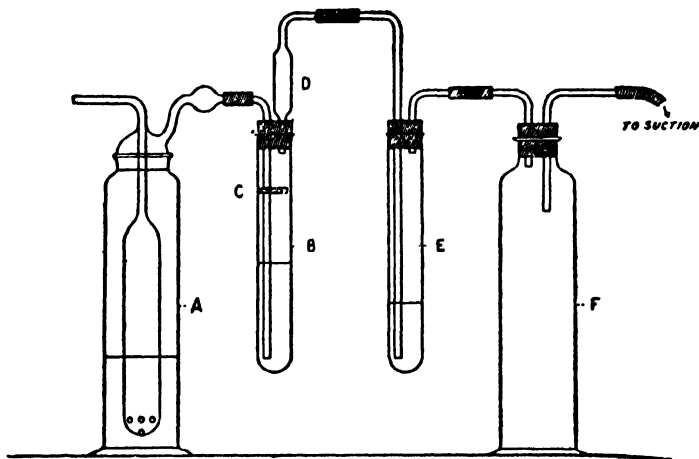


FIG. 41.—APPARATUS FOR DETERMINATION OF AMMONIA

#### 28.10 DETERMINATION

Introduce 2–4 g of the finely divided meat into tube *B* and add 20 ml of  $\text{NH}_3$ -free  $\text{H}_2\text{O}$ . Place measured quantity of 0.04 *N* or 0.02 *N*  $\text{H}_2\text{SO}_4$  or  $\text{HCl}$  in tube *E*. Add 1 ml of saturated K oxalate soln to sample in tube *B*, introduce a few drops of kerosene, and finally add just sufficient saturated Na or K carbonate soln to render mixture alkaline. Place tubes in position at once, pass air thru apparatus, and titrate the standard acid in tube *E* at hourly intervals until  $\text{NH}_3$  ceases to be given off,

using methyl red, cochineal, or congo red indicator. (If preferred, the  $\text{NH}_3$  collected in tube *E* may be determined by nesslerizing as directed under 37.11.)

#### NITRATES (INCLUDING ALSO NITRITES)

##### *Ferrous Chloride Method (5)—Tentative*

#### 28.11

##### REAGENTS

(a) *Ferrous chloride soln.*—Dissolve 400 g of nails, tacks, or other small pieces of Fe in 2 liter Florence flask with 1 liter of HCl, excluding air from flask by means of stopper equipped with Bunsen valve. When evolution of gas ceases, transfer, and keep the soln in completely filled 50 ml glass-stoppered bottles. Use only freshly opened bottles of the reagent.

(b) *Standard sodium nitrate soln.*—Dissolve 2 g of  $\text{NaNO}_3$  in 1 liter of recently boiled  $\text{H}_2\text{O}$ . Determine NO in 50 ml of this soln (equivalent to 0.1 g of  $\text{NaNO}_3$ ) as directed under 28.13.

#### 28.12

##### APPARATUS

Clamp to an Fe stand a 500 ml Kjeldahl flask fitted with 2-holed stopper. Thru one of holes pass stem of 100–125 ml cylindrical separator having a glass stopcock, and into other fit delivery tube leading downward at an angle from flask into trough containing a soln of commercial NaOH (1+1). Terminate upper end of delivery tube just below stopper in flask and place lower end, which is slightly constricted, bent upward, and covered with rubber tubing to prevent fracture, under surface of the NaOH soln in trough, the exit being just below mouth of an inverted measuring tube (50 ml plain eudiometer tube) filled with the NaOH soln. A single coil of Sn tubing fitted into trough and carrying current of cold  $\text{H}_2\text{O}$  greatly facilitates determination.

#### 28.13

##### DETERMINATION

Extract 100 g of the sample by boiling 6–7 times with successive 35–50 ml portions of  $\text{H}_2\text{O}$ , decant extracts thru muslin or paper filter into casserole, and evaporate combined extracts to volume of ca 50 ml. Introduce 50 ml of the  $\text{FeCl}_2$  soln and 50 ml of HCl (1+2.5) into Kjeldahl flask, close stopcock of separator, move end of delivery tube so that escaping air will not pass into measuring tube, and boil contents of flask until the air is completely expelled. Place exit end of delivery tube beneath measuring tube and boil the contents of the flask 1 min. longer to make certain that no air remains. Introduce 50 ml of standard  $\text{NaNO}_3$  soln into flask, a little at a time thru separator, continuously boiling contents of flask to force the NO gas into measuring tube. Finally rinse separator 3 or 4 times with 5–10 ml of recently boiled  $\text{H}_2\text{O}$ , adding rinsings to contents of evolution flask in manner described above. When evolution of gas ceases, cover opening of measuring tube with a porcelain crucible, using tongs, and carefully transfer tube to tall glass jar containing NaOH (1+1) soln, kept at room temp. The temp. of the surrounding caustic soln will soon (10–15 min.) be imparted to contents of the tube, and the volume of NO is read with tube in such a position that level of soln within tube coincides with level outside. Calculate percentage of nitrates and nitrites as  $\text{NaNO}_3$  from volume of NO obtained from sample compared with volume obtained from 0.1 g of  $\text{NaNO}_3$ , both measured under identical conditions.

After measuring tube has been removed, quickly insert over the delivery tube another tube filled with soln of commercial NaOH (1+1) and boil 1 min. longer to make sure that all the NO has been expelled. Run another 50 ml portion of standard

soln into apparatus and repeat determination. Then run sample in same manner, making certain that all the NO gas has been expelled and rinsing out both casserole and separator 3 or 4 times; 6 to 8 determinations may be made, excluding 2 standards. Finally run another standard. The 3 standards should check within 0.5 ml on 30–35 ml; 0.1 g of  $\text{NaNO}_3$  should give 26.36 ml of NO at  $0^\circ$  and 760 mm pressure. Report results as percentage of  $\text{NaNO}_3$ .

*Xylenol Method (6)—Tentative*

**28.14**

**APPARATUS**

Use simple distillation apparatus, including distillation bulb. A glass condenser of a type utilizing a thin, rapidly moving film of  $\text{H}_2\text{O}$  as cooling medium (West type) is recommended. Quickly remove any nitroxylenol solidifying in condenser by stopping flow of  $\text{H}_2\text{O}$  and allowing condenser to become warm.

**28.15**

**REAGENTS**

(a) *Meta-xylenol*.—1-hydroxy, 2,4-dimethylbenzene. Eastman's preparation No. 1150, or equivalent.

(b) *Silver ammonium hydroxide soln*.—Dissolve 5 g of nitrate-free  $\text{Ag}_2\text{SO}_4$  in 60 ml of  $\text{NH}_4\text{OH}$ . Heat mixture to boiling, concentrate to ca 30 ml, cool, and dilute to 100 ml with  $\text{H}_2\text{O}$ .

(c) *Bromocresol green indicator*.—Dissolve 0.1 g of bromocresol green in 1.5 ml of 0.1 *N* NaOH, and make up to 100 ml with  $\text{H}_2\text{O}$ .

(d) *Standard nitrate soln*.—Dissolve 0.1804 g of recrystallized  $\text{KNO}_3$  in  $\text{H}_2\text{O}$  and make up to 1 liter, or dilute 17.85 ml of 0.1 *N*  $\text{HNO}_3$  to 1 liter. 10 ml contains 0.25 mg of nitrate nitrogen.

**28.16**

**DETERMINATION**

Mix 5–10 g of the finely comminuted and thoroly mixed sample with 80 ml of warm  $\text{H}_2\text{O}$ . Break up all lumps and heat on steam bath for 1 hour, stirring occasionally. Transfer to 100 ml volumetric flask, cool, make to mark, and mix. Filter, or allow to settle, and pipet 40 ml of filtrate, or supernatant liquid, into 50 ml volumetric flask. (No correction for volume occupied by the meat is necessary.) Add 3 drops of the bromocresol green indicator. Add  $\text{H}_2\text{SO}_4$  (1+10) dropwise until color changes to yellow. Oxidize nitrites to nitrates by adding 0.2 *N*  $\text{KMnO}_4$  soln dropwise with shaking until faint pink color remains 15–30 seconds. Add 1 ml of  $\text{H}_2\text{SO}_4$  (1+10) and 1 ml of phosphotungstic acid soln (20 g in 100 ml). Make to mark, mix, and filter.

Measure into 500 ml flask (Erlenmeyer is satisfactory) an aliquot (not more than 20 ml) containing from 0.005 to 0.15 mg of nitrate N. (If more than 20 ml is required, make slightly alkaline and concentrate by evaporation.) Add sufficient quantity of the Ag ammonium hydroxide soln to precipitate all chlorides and most of excess phosphotungstic acid. (A slight excess of the Ag reagent is not harmful; 1 or 2 ml is usually sufficient.) Without decanting or filtering, add volume of  $\text{H}_2\text{SO}_4$  (3+1) ca three times volume of liquid in flask. Stopper flask, mix, cool to ca  $35^\circ$ , add 0.05 ml (1–2 drops) of the m-xylenol, stopper, shake, and hold at  $30\text{--}40^\circ$  for 30 min.

(A yellow to brownish yellow color, indicative of nitrates, will appear. A bright red precipitate, due to incomplete removal of phosphotungstic acid, may also appear. A slight excess of phosphotungstic acid causes no interference but a large excess may do so.)

After nitration is complete, add 150 ml of  $H_2O$ , taking care to wash off stopper, and distil until 40–50 ml has passed over into receiver containing 5 ml of NaOH (10 g/liter). Transfer distillate to 100 ml volumetric flask, make up to volume with  $H_2O$ , and determine nitrate N by comparing color of suitable aliquot with set of graded color standards containing 0.003–0.006 mg of nitrate N.

Prepare the color standard from 10 ml of the nitrate standard as directed previously, using 0.05 ml of the m-xyleneol and 30 ml of  $H_2SO_4$  (3+1), and making up distillate to 500 ml. Prepare color standard fresh each day, as it becomes cloudy on standing.

**28.17****NITRITES (7)—TENTATIVE**

(Applicable to cured meats)

Weigh 5 g of finely comminuted and thoroly mixed sample into 50 ml beaker. Add ca 40 ml of nitrite-free  $H_2O$  heated to 80°. Mix thoroly by stirring with glass rod, taking care to break up all lumps, and transfer to 500 ml graduated flask. Wash out beaker and rod thoroly with successive portions of the hot  $H_2O$ , adding all washings to flask. Add sufficient hot  $H_2O$  to bring contents of flask to volume of ca 300 ml, transfer flask to steam bath, and let stand 2 hours, shaking occasionally. Add 5 ml of saturated  $HgCl_2$  soln and mix. Cool to room temp., make to mark with nitrite-free  $H_2O$ , and mix again. Filter, and determine nitrite N in suitable aliquot as directed under 37.15, reporting results as p.p.m. of  $NaNO_2$ .

**STARCH**

(In chopped meat, sausage, deviled meat, etc.)

**28.18***Qualitative Test—Tentative*

Treat 5–6 g of sample with boiling  $H_2O$  for 2–3 min., cool mixture, and test supernatant liquid with I soln, 33.30(f). (In interpreting this test it should be remembered that a small quantity of starch may be present as result of use of spices. If marked reaction is given, however, it may be concluded that starch or flour has been added, and a quantitative determination should be made. The qualitative test may be replaced by microscopic examination, which discloses not only presence of added starch but also variety used.)

**28.19***Quantitative Method (8)—Tentative*

Treat in 250 ml beaker 10 g of finely divided sample with 75 ml of 8% soln of KOH in alcohol and heat on steam bath until all meat is dissolved (30–45 min.). Add equal volume of alcohol, cool, and allow to stand at least an hour. Filter by suction thru thin layer of asbestos in Gooch crucible. Wash twice with warm 4% soln of KOH in alcohol, 50% by volume, and then twice with warm 50% alcohol. Discard washings. Retain as much of precipitate in beaker as possible until last washing. Place crucible with contents in original beaker and add 40 ml of  $H_2O$  and 25 ml of  $H_2SO_4$ . Stir during addition of acid and make sure that it comes in contact with all the precipitate. Allow to stand ca 5 min., add 40 ml of  $H_2O$ , and heat just to boiling, stirring constantly. Transfer soln to 250 ml volumetric flask, add 2 ml of 20% phosphotungstic acid soln, allow to cool to room temp., and make up to mark with  $H_2O$ . Filter thru starch-free filter paper, pipet 100 ml of filtrate into 200 ml volumetric flask, neutralize with NaOH (1+1), make to volume, and determine dextrose present in 50 ml portion of filtrate as directed under 34.39, titrat-

ing the  $\text{Cu}_2\text{O}$  precipitate as directed under 34.42. Weight of dextrose  $\times 0.9$  = weight of starch.

**28.20****SOYBEAN FLOUR—QUALITATIVE TEST (9)—TENTATIVE**

Treat 10 g of meat with 8% alcoholic KOH as directed in 28.19. As soon as all of meat has disintegrated, transfer liquid and residue to 100 ml graduated sedimentation tube, make up to 100 ml with alcohol, and allow to settle. Decant off supernatant liquid as completely as possible, and cover residue with ca 50 ml of warm  $\text{H}_2\text{O}$ . Stopper tube and shake vigorously; allow to stand a few min. until foam subsides, then transfer to 50 ml centrifuge tube, and centrifuge. Pour off and discard supernatant liquid and add 10 ml of HCl to centrifuge tube. Stopper and shake, or mix contents thoroly with glass rod. Add ca 15 ml of 25% alcohol, and after mixing, centrifuge. Pour off supernatant liquid and examine residue under microscope for characteristic "hour-glass" or I-shaped cells (sometimes called "bearer cells"), preferably with use of polarized light.

**GLYCOGEN****28.21***Qualitative Test (10)—Tentative*

Boil 50 g of the macerated sample with 50 ml of  $\text{H}_2\text{O}$  for 15–30 min. Filter broth thru moistened filter paper or fine linen. To portion of filtrate in test tube add a few drops of a mixture of 2 parts of I, 4 parts of KI, and 100 parts of  $\text{H}_2\text{O}$ . If a considerable quantity of glycogen is present, it produces a dark brown color; this color is destroyed by heating, but it reappears on cooling. If starch is present, it may be precipitated by treating the water extract with two volumes of acetic acid and after filtering applying test for glycogen to filtrate.

*Quantitative Method (11)—Tentative***28.22****PREPARATION OF SOLUTION**

Weigh by difference ca 25 g of the finely ground and thoroly mixed sample. Place in 400 ml beaker and mix with 50 ml of KOH soln (1.5+1), free from carbonate. Cover beaker with watch-glass and digest on steam bath for 2 hours, stirring occasionally. Dilute to ca 200 ml with cold  $\text{H}_2\text{O}$ .

**28.23****DETERMINATION**

Add to the soln, 28.22, an equal volume of alcohol, cover with watch-glass, and set aside for 10–12 hours. Decant supernatant liquid thru folded 18.5 cm filter, allowing glycogen to remain in beaker, and wash by decantation with alcohol (2+1) until glycogen is white, or nearly so. (Usually ca 4 washings are required.) Transfer washed precipitate from beaker to filter and wash 2 or 3 times with the dilute alcohol. (Soln filters slowly, and funnel should be covered with watch-glass to prevent excessive evaporation. The albuminous substance present retards filtration if it is permitted to dry on the paper. If washing by decantation is not made as complete as possible, it will be difficult to obtain the glycogen free from the coloring matter.)

After washing is completed, close bottom of funnel by piece of rubber tubing and pinch-cock. Fill funnel with warm  $\text{H}_2\text{O}$ , cover with watch-glass, and let stand 2–3 hours, or overnight. Open pinch-cock and allow all the soln to pass thru filter into beaker. Close funnel with the pinch-cock and fill with warm  $\text{H}_2\text{O}$  as before. Allow this  $\text{H}_2\text{O}$  to remain in funnel for 1 hour and then filter as before. At first the glycogen soln appears quite turbid. Continue washing with warm  $\text{H}_2\text{O}$  until filtrate becomes

perfectly clear. To the soln of glycogen in  $H_2O$ , add double its volume of alcohol and let stand overnight to complete the reprecipitation of the glycogen. Filter, and wash as before with alcohol (2+1).

If desired, the last filtration may be made thru a weighed Gooch crucible and the weight of glycogen determined after drying to constant weight. This gives results that are approximately correct. More satisfactory results are obtained by hydrolyzing the glycogen with  $HCl$  (1+3) and determining the resultant dextrose. Dissolve the glycogen on filter in warm  $H_2O$  as directed above, collecting filtrate and washings in 300 ml volumetric flask and keeping volume within 225 ml. Add 12.5 ml of  $HCl$  to combined filtrate and washings, mix, and place in boiling water bath for 3 hours. Cool, neutralize with 10%  $NaOH$  soln, cool again, make up to volume with  $H_2O$ , and determine dextrose in aliquot of the soln as directed under 34.39, determining reduced  $Cu$  as directed under 34.42. Corresponding weight of dextrose  $\times 0.9$  = its equivalent of glycogen. Correct this result for dilution to obtain percentage of glycogen in sample.

**28.24****DEXTROSE—TENTATIVE**

Weigh 100 g of the finely ground sample into 600 ml beaker, add 200 ml of  $H_2O$ , heat to boiling, and boil gently for 5 min. Stir contents of beaker frequently during this and subsequent extractions to prevent bumping. (When several samples are extracted at same time a mechanical stirring device is practically a necessity.) Remove beaker from flame, allow insoluble matter to settle, and decant clear liquid on asbestos mat in a 4" funnel. Filter with aid of suction. Add 150 ml of hot  $H_2O$  to residue in beaker, boil gently for 5 min., let settle, and decant clear liquid as directed previously. Repeat operation, finally transfer contents of beaker to funnel, wash with 150–200 ml of hot  $H_2O$ , and press meat residue as dry as possible. Transfer contents of filter flask to evaporating dish and evaporate on steam bath to volume of ca 25 ml but not to dryness. Transfer extract to 100 ml volumetric flask, taking care that volume of liquid does not exceed 60 ml. Add 25–35 ml of phosphotungstic acid soln (1+1), shake vigorously, let stand a few minutes for gas bubbles to rise to surface, make to volume, shake, and either filter or centrifuge. (Use of centrifuge is to be preferred, because a larger volume of liquid is obtained.) Test a portion of filtrate with dry phosphotungstic acid for complete precipitation. If appreciable precipitate forms, take an aliquot of filtrate, add 5–10 ml of the phosphotungstic acid soln, make to volume, filter, and test filtrate for complete precipitation. Filtrate should also show not more than slight reaction for creatinin when tested by adding to 5 ml a few drops of saturated aqueous picric acid soln and making mixture alkaline with a few drops of 10%  $NaOH$  soln (12).

Transfer 50 ml of clarified extract to 100 ml volumetric flask, add 5 ml of  $HCl$ , and invert soln as directed under 27.33. Cool soln, neutralize to litmus, cool, make to volume, and filter. To filtrate add sufficient dry powdered  $KCl$  to precipitate excess phosphotungstic acid, filter, test filtrate for complete precipitation, and determine reducing sugar as directed under 34.35 or 34.39, ascertaining quantity of reduced  $Cu$  as directed under 34.44. Calculate total sugar as dextrose from 44.12.

If an abnormal reduction is obtained when clarified meat extract is boiled with Fehling soln, i.e., if soln turns yellow, brown, green, or muddy in appearance instead of reddish-blue, discard determination, since incomplete precipitation of nitrogenous compounds, due to use of insufficient phosphotungstic acid, is indicated.

**28.25****PRESERVATIVES—OFFICIAL.—See Chap. 32****28.26****METALS—TENTATIVE.—See Chap. 29**



## 28.27

COLORING MATTERS—TENTATIVE.—*See* Chap. 21

## SOLUBLE AND INSOLUBLE NITROGEN—TENTATIVE

## 28.28

## PREPARATION OF SOLUTION

Exhaust 7–25 g of the sample (depending upon  $H_2O$  content) in following manner: Weigh into 150 ml beaker, add 5–10 ml of cold ( $15^\circ$ )  $NH_3$ -free  $H_2O$ , and stir to homogeneous paste. Add 50 ml of cold  $H_2O$ , stir for 15 min. at 3 min. intervals, let stand for 2–3 min., and decant liquid thru quantitative filter, collecting filtrate in 500 ml volumetric flask. Drain beaker, pressing out liquid from meat residue by aid of glass rod. Add to residue in beaker 50 ml of cold  $H_2O$ , stir for 5 min., allow to stand 2–3 min., and decant as before. If a considerable portion of meat is transferred to filter, return it to beaker by means of glass rod. Repeat extractions, using two 50 ml portions and four 25 ml portions of cold  $H_2O$ . After the last extraction transfer entire insoluble portion to filter and wash with three 10 ml portions of  $H_2O$ , allowing material to drain thoroly after each addition of  $H_2O$ . Dilute to mark and mix thoroly.

## 28.29

## DETERMINATION

Determine soluble N in 50 ml aliquot of soln obtained, 28.28, proceeding as directed under 2.24, 2.25, or 2.26. Total N—soluble N=insoluble N.

## 28.30

## COAGULABLE NITROGEN—TENTATIVE

(For uncooked meat only)

Measure 150 ml of extract, 28.28, into 250 ml beaker and evaporate to 40 ml on steam bath, stirring occasionally. Neutralize to phenolphthalein, using the indicator outside the soln to avoid subsequent interference in the determination of creatin, 28.33. Add 1 ml of 0.1 *N* acetic acid, and boil gently for 5 min. (Coagulum should separate at once, leaving clear liquid.) Filter thru quantitative paper and wash beaker thoroly 4 times with hot  $H_2O$ , taking special care to clean sides. Finally wash coagulum on filter 3 times, dilute combined filtrate and washings to definite volume, and reserve for determination of proteose, peptone, and gelatin, 28.31, and creatin, 28.33. Transfer coagulum with paper to Kjeldahl flask and remove, with  $H_2SO_4$ , any of material adhering to beaker, taking the usual 25 ml of acid in 5 ml portions for this purpose, heating acid in beaker on hot plate, and rubbing with glass rod. Proceed as directed under 2.24, 2.25 or 2.26.

## PROTEOSE, PEPTONE, AND GELATIN NITROGEN

## 28.31

*Modified Tannin-Salt Method (13)—Tentative*

Transfer 50 ml aliquot of filtrate, 28.30, to 100 ml volumetric flask, add 15 g of NaCl and 10 ml of cold  $H_2O$ , shake until the NaCl has dissolved, and cool to  $12^\circ$ . Add 30 ml of 24% tannin soln cooled to  $12^\circ$ , dilute to mark with  $H_2O$  previously cooled to  $12^\circ$ , shake, and allow mixture to stand at temp. of  $12^\circ$  for 12 hours, or overnight. Filter at  $12^\circ$ , transfer 50 ml of filtrate to Kjeldahl flask, and add a few drops of  $H_2SO_4$ . Place flask in steam bath, connect with vacuum pump, and evaporate to dryness. Determine N in residue as directed under 2.24, using 30 ml of  $H_2SO_4$  for the digestion. Conduct blank determination, using same quantity of reagents, and correct result accordingly. Multiply corrected result by 2 and deduct quantity of N found from N determined in another 50 ml aliquot of filtrate, 28.30,

that has not been treated with tannin-NaCl. Difference  $\times 6.25$  = percentage of proteose, peptone, and gelatin.

**28.32****MEAT BASES—TENTATIVE**

Deduct from percentage of total N, **28.8**, the sum of percentages of N obtained in determination of insoluble N, **28.29**, coagulable N, **28.30**, and proteose, peptone, and gelatin, **28.31**, to obtain percentage of N of meat bases. Multiply result by 3.12 to obtain percentage of meat bases.

**28.33****CREATIN—OFFICIAL**

Evaporate aliquot or remaining portion of filtrate and washings from coagulable N, **28.30** (a portion having been used in **28.31**), to 5–10 ml; transfer with minimum quantity of hot  $H_2O$  to 50 ml volumetric flask, keeping volume below 30 ml; add 10 ml of 2  $N$  HCl; and mix. Hydrolyze in autoclave at  $117\text{--}120^\circ$  for 20 min., allow flask to cool somewhat, remove, and chill under running  $H_2O$ . Partially neutralize excess of acid by adding 7.5 ml of 10% NaOH soln (free from carbonates), dilute to mark, and mix. Make preliminary reading on 20 ml with Duboseq colorimeter to ascertain volume to use to obtain a reading of ca 8 mm. Transfer such a volume of the soln to a 500 ml volumetric flask and add 10 ml of 10% NaOH soln and 30 ml of saturated picric acid soln (1.2%). Mix, rotate 30 seconds, and let stand exactly 4.5 min. Dilute to mark at once with  $H_2O$ , shake thoroly, and compare, preferably in a Duboseq colorimeter, with a standard soln prepared by treating 50 ml of a soln of creatinin zinc chloride in 0.1  $N$  HCl (1.603 g/liter—0.001 g creatinin/ml) with NaOH and picric acid, and making up to 500 ml in manner described above.

**AMINO NITROGEN***Van Slyke Method (14)—Tentative***28.34****APPARATUS**

Use apparatus shown in Figs. 42 and 43, the former illustrating manner in which entire apparatus is arranged and the latter showing details of the deaminizing bulb and connections. The Hempel gas pipet is filled with a soln containing 50 g of  $KMnO_4$  and 25 g of KOH/liter.

**28.35****DETERMINATION**

Fill with  $H_2O$  buret (*F*), capillary tube leading to Hempel pipet, and also other capillary as far as *c*. Introduce into *A* sufficient acetic acid to fill  $\frac{1}{2}$  of *D*, etching tube *A* with mark to measure this quantity. Allow acid to run into *D*, and turn cock *c* so as to allow air to escape from *D*. Pour  $NaNO_2$  soln (300 g/liter) into *A* until *D* is filled and enough excess is present to rise a little above the cock into *A*. *A* is also marked for measuring off this quantity. Close gas exit from *D* at *c*, and, *a* being open, shake *D* a few seconds until liquid is forced down to 20 ml mark in *D*. Close *a*, open *c*, and shake apparatus rapidly with motor for 2 min., these operations being for purpose of expelling all air from *D*. Turn *c* and *f* so that *D* and *F* are connected.

Measure off in *B* 10 ml or less, as case may be, of the soln of the sample containing not more than 20 mg of amino N (1–2 g of the sample in the case of meat extracts) and allow it to run into *D*. Connect *D* with motor as shown in Fig. 42 and shake for 5 min.

If the soln of sample is viscous and threatens to foam over, rinse out *B*, and thru it introduce a little capryl alcohol into *D*, or if it is known beforehand that sample

will cause excessive foaming, introduce a little capryl alcohol into *D* thru *B*, rinsing *B* with alcohol and ether or drying with roll of filter paper before adding soln of sample.

During the shaking there is evolution of *N* mixed with *NO*, the gases being

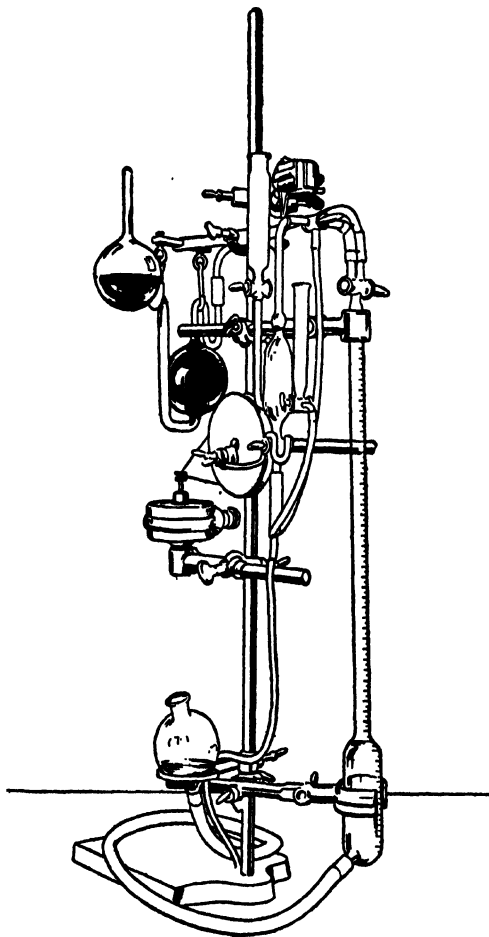


FIG. 42.—VAN SLYKE APPARATUS FOR DETERMINATION OF AMINO NITROGEN

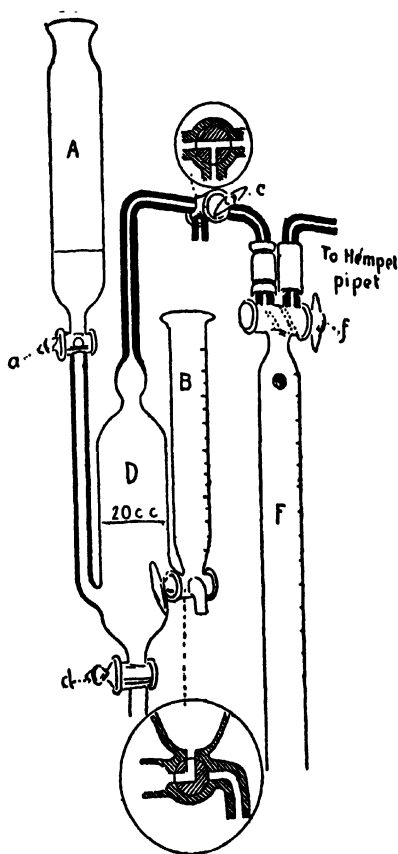


FIG. 43.—DETAILS OF DEAMINIZING BULB AND CONNECTION

collected in *F*. Force all the gas in *D* into *F* by opening *a* and filling *D* with liquid from *A*. Connect *F* with Hempel pipet and force the gas into latter by means of leveling bulb, allowing cock *a* to remain open during this and succeeding operation in order to permit displacement of the liquid in *D* by the *NO* formed in interval. Connect driving rod with pipet by lifting hook from shoulder of *D* and placing other hook, on opposite side of driving rod, over horizontal lower tube of pipet. Shaking pipet rather slowly for a few minutes completes absorption of *NO* except with al-

most completely exhausted  $\text{KMnO}_4$  solns. Return gas to buret and adjust level with leveling bulb; note volume of N, temp., and barometric pressure, and calculate volume of N under standard conditions of temp. and pressure. Obtain corresponding weight of N, divide latter by 2, and from quotient calculate apparent percentage of amino N in sample. Correct result for blank test performed as above, using 10 ml of  $\text{H}_2\text{O}$  instead of the soln of the sample. The quantity of gas obtained in blank is usually 0.3–0.4 ml, and nitrite solns giving a much larger correction should be rejected.

With beef extracts and similar preparations, 5 min. is sufficient time to allow for completion of reaction in *D*. In general, the same time serves for decomposition of  $\alpha$ -amino acids, but with ammonia, methylamine, and most amines other than  $\alpha$ -amines 1–1.5 hours should be allowed. For determinations on such substances mix soln of sample with the reagents, as described previously, allow mixture to stand in apparatus till end of required time, and conclude reaction by shaking apparatus with the motor for 2–3 min. Continue determination as directed previously.

**28.36***Sørensen Method (15)—Tentative*

To 20 ml of filtrate, **28.30**, neutralized to phenolphthalein with  $\text{Ba}(\text{OH})_2$  or  $\text{NaOH}$ , or to 20 ml of equivalent extract of the meat (in some cases larger volume may be necessary) add 10 ml of freshly prepared phenolphthalein-formol mixture (50 ml of 40% formaldehyde soln containing 1 ml of a 0.5% soln of phenolphthalein in 50% alcohol, exactly neutralized with 0.2 *N*  $\text{Ba}(\text{OH})_2$  or  $\text{NaOH}$ ). Titrate mixture with 0.2 *N*  $\text{Ba}(\text{OH})_2$  soln until distinct red color appears, add slight known excess of 0.2 *N*  $\text{Ba}(\text{OH})_2$ , and titrate back to neutrality with 0.2 *N*  $\text{HCl}$ . Conduct blank titration with same reagents, using 20 ml of  $\text{H}_2\text{O}$  in place of soln to be tested. From quantity of 0.2 *N*  $\text{Ba}(\text{OH})_2$  required to neutralize the mixture, corrected for quantity used in blank titration, calculate quantity of amino N present (including  $\text{NH}_3$  if this has not been removed). 1 ml of 0.2 *N*  $\text{Ba}(\text{OH})_2$  soln = 2.8 mg of amino N.

**28.37****TOTAL SOLUBLE PHOSPHORUS—TENTATIVE**

Evaporate to dryness 50 ml of the prepared water extract, **28.28**, moisten residue with 10 ml of  $\text{H}_2\text{SO}_4$ , add a few drops of  $\text{HNO}_3$ , and heat on hot plate until all organic matter is destroyed. Add 100 ml of  $\text{H}_2\text{O}$ , boil for a few minutes, and proceed as directed under **2.9**.

**28.38 SEPARATION OF SOLUBLE INORGANIC AND ORGANIC PHOSPHORUS—TENTATIVE**

To 500 ml of the prepared extract, **28.28**, add 50 ml of magnesia mixture, **2.7(c)**, and stir thoroly. Allow to stand 15 min., add 25 ml of  $\text{NH}_4\text{OH}$ , cover, and allow to stand 3 days. Filter, and wash precipitate with  $\text{NH}_4\text{OH}$  (1+9). Dissolve precipitate on filter paper and that remaining in beaker in  $\text{HNO}_3$  (1+1) and hot  $\text{H}_2\text{O}$ , receiving soln in 400 ml beaker. Neutralize with  $\text{NH}_4\text{OH}$ , make slightly acid with  $\text{HNO}_3$ , add 5 g of  $\text{NH}_4\text{NO}_3$ , and determine inorganic P as directed under **2.9**.

**AGAR AGAR—QUALITATIVE TEST—OFFICIAL****28.39****REAGENTS**

- (a) *Trichloroacetic acid soln*.—25 g of acid plus 50 ml of  $\text{H}_2\text{O}$ .
- (b) *Iodine soln*.—Approximately *N*/30.
- (c) *Benedict qualitative soln*.—See **22.138(a)**.

## 28.40

## PREPARATION OF SAMPLE

*Boned chicken or meat.*—Chill overnight in refrigerator to jell the broth. Separate, by means of thin-bladed spatula, as much jell as possible; warm on steam bath until it liquefies completely.

*Consommé or broth.*—No preparation necessary.

## 28.41

## DETECTION OF GUM

Transfer up to 40 ml of the liquefied jell from the meat, or 40 ml of consommé, to 100 ml beaker. Add 5 ml of the trichloroacetic acid soln, stir, and let stand 15–30 min. Transfer to 50 ml conical centrifuge tube and whirl 15–20 min. at ca 1200 r.p.m. Decant the clear supernatant layer into a 250 ml (8 oz.) centrifuge bottle or nursing bottle, add 4–5 volumes of alcohol, and allow to stand until a precipitate coagulates, or overnight. (No precipitate indicates absence of gums.) Whirl at 1200 r.p.m. 15–30 min. until precipitate packs to bottom of centrifuge bottle. Carefully decant the alcohol, taking care not to disturb the packed gum precipitate. Remove the few remaining drops of alcohol by spontaneous drying or by a gentle air blast. Add one drop of the *N*/30 I soln. An evanescent violet or black color indicates presence of agar agar. (Negative test does not necessarily mean agar agar is not present.)

Add 3 ml of hot  $H_2O$  and warm on steam bath until gum precipitate dissolves. Chill gum soln in mixture of ice and  $H_2O$ . A thickening, or a stiff jell, indicates agar agar. Warm cooled mixture on steam bath, transfer to 50 ml beaker, rinse centrifuge bottle with 3–4 ml of  $H_2O$ , and add rinsings to jell soln. Add 1 ml of  $HCl$  and boil 30 seconds. Transfer 1 ml of hydrolyzed gum soln to test tube, neutralize with 10%  $NaOH$  soln, using litmus paper as indicator (ca 2 ml required), remove litmus paper, add 5 ml of the Benedict soln, and boil cautiously over free flame 30–60 seconds. Green, yellow, or brick-colored precipitate after spontaneous cooling indicates agar agar (or other hydrolyzable gum).

## MEAT EXTRACTS AND SIMILAR PRODUCTS

## 28.42

## PREPARATION OF SAMPLE—OFFICIAL

Remove liquid and semi-liquid meat extracts and similar preparations from container and mix thoroly. (A little heating expedites mixing of pasty extracts.) Carefully remove sediment that forms in many liquid preparations from bottom of container and include in sample. If sample is in form of cubes, grind 10–12 of the cubes in mortar.

## 28.43

## MOISTURE—OFFICIAL

Proceed as directed under 27.3, using ca 2 g of powdered preparations, ca 3 g of pasty preparations, and 5–10 g of liquid extracts, according to solid content. Dry the powdered preparations directly without admixture. Dissolve pasty preparations in  $H_2O$  and dry with sufficient ignited sand, asbestos, or pumice stone to absorb the soln. When glycerol is present, proceed as directed under 27.7.

## 28.44

## ASH—OFFICIAL

Proceed as directed under 34.9 or 34.10. Add sufficient  $H_2O$  to pasty preparations to effect soln and evaporate to dryness in order that solids may be distributed evenly over bottom of dish.

## 28.45

## TOTAL PHOSPHORUS—OFFICIAL

Destroy organic matter as directed under 2.8(c) or (d), and proceed as directed under 2.9 or 2.12.

28.46

## CHLORIDES—OFFICIAL

Dissolve ca 1 g of prepared sample, 28.42, in 20 ml of 5%  $\text{Na}_2\text{CO}_3$  soln and proceed as directed under 12.41 and 12.42.

28.47

## FAT—TENTATIVE

Transfer residue from determination of moisture to continuous extraction apparatus and proceed as directed under 27.25.

28.48

## TOTAL NITROGEN—OFFICIAL.—See 2.24, 2.25, or 2.26.

28.49

## AMMONIA—TENTATIVE

Introduce 1 g of pasty extracts or 2–3 g of fluid extracts into tube *B* of the Folin apparatus and proceed as directed under 28.10.

28.50

## INSOLUBLE NITROGEN (16)—TENTATIVE

Dissolve in cold  $\text{H}_2\text{O}$  5 g of powdered preparations, 8–10 g of pasty extracts, and 20–25 g of fluid extracts. Filter, and wash with cold  $\text{H}_2\text{O}$ . Transfer filter paper and contents to Kjeldahl flask and determine N as directed under 2.24, 2.25, or 2.26. If a large quantity of insoluble matter is present, transfer the weighed sample to volumetric flask, dilute to definite volume, shake thoroly, filter thru folded filter, and determine N in aliquot of filtrate. Total N, 28.48,  $-\text{N}$  in total filtrate = insoluble N. Insoluble N  $\times 6.25$  = percentage of insoluble protein.

28.51

## COAGULABLE NITROGEN—TENTATIVE

Use as large an aliquot of the filtrate from the insoluble N, 28.50, as practicable' and neutralize to phenolphthalein by addition of acetic acid or  $\text{NaOH}$ , whichever may be necessary; add 1 ml of 1 *N* acetic acid, boil 2–3 min., cool to room temp., dilute to 500 ml, and pass thru folded filter.

Determine N in 50 ml of filtrate as directed under 2.24, 2.25, or 2.26. Soluble N (total N – insoluble N)  $- 10 \times$  the N obtained = percentage of N present as coagulable N. Coagulable N  $\times 6.25$  = coagulable protein in sample.

28.52

## PROTEOSES AND GELATIN (17)—TENTATIVE

Evaporate filtrate from 28.51 to small volume and saturate with  $\text{ZnSO}_4$  (ca 85 g to 50 ml, avoiding such an excess as would later cause bumping). Let stand several hours, filter, and wash precipitate with saturated  $\text{ZnSO}_4$  soln. Place filter and precipitate in Kjeldahl flask and determine N as directed under 2.24, 2.25, or 2.26. Or, if precipitate is voluminous, which is unusual, dilute to definite volume with saturated  $\text{ZnSO}_4$  soln, filter, and determine N in aliquot of filtrate as directed under 2.24, 2.25, or 2.26. N in filtrate from coagulable N, 28.51,  $-\text{N}$  thus obtained = N of precipitated protein (proteoses and gelatin).

28.53

## GELATIN—TENTATIVE

Prepare 50% soln of sample, using hot  $\text{H}_2\text{O}$ , allow to cool, and place in ice box for 2 hours. If gelatin is present, the soln will set.

The ratio of total creatinin to total N in normal meat extract (1:1.5) assists in determining presence of gelatin or gelatin derivatives. The ratio is decreased when gelatin or gelatin derivatives are present in any considerable quantity.

28.54

## AMINO NITROGEN—TENTATIVE

Proceed as directed under 28.35 or 28.36, using aliquot of filtrate from 28.51.

**28.55 ACID ALCOHOL-SOLUBLE NITROGEN (18)—TENTATIVE**

Transfer 10 ml of an aqueous soln of sample (10 g of sample dissolved in sufficient H<sub>2</sub>O to make 100 ml), or, if sample is insoluble in H<sub>2</sub>O, 1 g of sample and 10 ml of H<sub>2</sub>O, to 200 ml glass-stoppered measuring cylinder; add 1.2 ml of 12% HCl, mix, and add absolute alcohol to 200 ml mark. Mix thoroly and set aside for several hours. If necessary, make to volume, filter, transfer 100 ml of filtrate to Kjeldahl flask, evaporate alcohol on water bath, and determine N in residue as directed under 2.24, 2.25, or 2.26.

**28.56 CREATIN—OFFICIAL**

Dissolve ca 7 g of sample in cold (20°) NH<sub>3</sub>-free H<sub>2</sub>O in 150 ml beaker, transfer soln to 250 ml volumetric flask, dilute to mark, and mix thoroly. Transfer 20 ml aliquot of this soln to 50 ml volumetric flask and proceed as directed under 28.33. Subtract from combined creatinin value the equivalent of pre-formed creatinin, 28.57, and multiply difference by 1.16 to convert into creatin. Express result as percentage of creatin.

**28.57 CREATININ—OFFICIAL**

Measure ca 5 ml of soln used in 28.56 into 500 ml volumetric flask, add 10 ml of 10% NaOH soln and 30 ml of saturated picric acid soln (1.2%), mix, and rotate for 30 seconds. Allow to stand exactly 4.5 min. and then dilute to mark at once with H<sub>2</sub>O. Shake thoroly and read depth of color after standing. If reading is less than 7 or more than 9.5 mm, repeat, calculating quantity of soln necessary to obtain reading of ca 8 mm. Express result as percentage of creatinin, making calculations as indicated under 28.33.

**28.58 NITRATES (INCLUDING ALSO NITRITES)—TENTATIVE.—See 28.13 or 28.16****28.59 GLYCEROL (19)—TENTATIVE**

Weigh 2 g of a solid or 5 g of a liquid preparation in small Pb dish or thin glass shell containing 20 g of ignited sand. Transfer dish and contents to mortar containing more ignited sand and several grams of anhydrous Na<sub>2</sub>SO<sub>4</sub> and mix thoroly. Transfer mixture, including dish, to Soxhlet apparatus that has a piece of cotton placed in side arm to prevent solid particles from being siphoned over. Extract entire mass with redistilled anhydrous acetone for 10 hours. Distil acetone from extract, carefully removing last trace by means of vacuum pump. Take up residue in H<sub>2</sub>O, add 5 ml of 10% AgNO<sub>3</sub> soln, dilute to volume of 100 ml, shake, allow to stand overnight, filter, and determine glycerol in aliquot of filtrate as directed under 33.76, 5th par., beginning "Add 1 ml of H<sub>2</sub>SO<sub>4</sub>." With solid meat and yeast extracts a blank of 0.5–1.0% is obtained in most cases.

**28.60 SUGAR—TENTATIVE**

Heat 20 g of sample with ca 200 ml of H<sub>2</sub>O on steam bath until all soluble substances have gone into soln, and proceed as directed under 28.24. Reducing sugars to extent of 0.5% may be present as natural constituent of meat extracts.

**28.61 PRESERVATIVES—OFFICIAL.—See Chap. 32****28.62 METALS—TENTATIVE.—See Chap. 29****SULFUR DIOXIDE****28.63 Distillation Method.—See 32.32**

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## 29. METALS, OTHER ELEMENTS AND RESIDUES IN FOODS

### ARSENIC (1)

#### *Gutzeit Method—Official*

#### 29.1

#### REAGENTS

(a) *Stannous chloride soln.*—Dissolve 40 g of As-free  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  in HCl and make up to 100 ml with the same strength acid.

(b) *Zinc.*—Use 20- or 30-mesh, As-free granulated Zn, that needs no preliminary treatment, or As-free stick Zn either cut into equal 1 cm lengths, or melted and cast into pellets in porcelain mold drilled (for example) 9 mm in diam. and 12.5 mm deep. Activate the pieces of Zn with HCl (1+3), to which has been added 2 ml of the  $\text{SnCl}_2$  soln, allowing action to continue 15 min. Sort out distinctly inactive or overactive pieces and pour off liquid. Wash Zn free from acid with clear tap  $\text{H}_2\text{O}$ , and rinse with hot  $\text{H}_2\text{O}$ . Select uniformly etched non-pitted Zn and store in suitable receptacle. To maintain supply of uniform Zn adopt a system of rotation by withdrawing Zn from original receptacle until stock is exhausted and storing used Zn in second receptacle after discarding non-uniform or deeply pitted pieces. Draw Zn from second receptacle after washing it with clear running  $\text{H}_2\text{O}$ . Repeat procedure until pieces are too small for further use.

(c) *Ammonium oxalate soln.*—Saturated.

(d) *Potassium iodide soln.*—Dissolve 15 g of KI in  $\text{H}_2\text{O}$  and dilute to 100 ml.

(e) *Sand.*—Clean 30-mesh (thru 30- but not 40-mesh) white sea sand by washing successively with hot 10% NaOH soln, hot  $\text{HNO}_3$ , and hot distilled  $\text{H}_2\text{O}$ . Dry the clean sand.

(f) *Mercuric bromide paper.*—Use commercial As papers cut from paper of uniform weight and texture into strips exactly 2.5 mm wide and ca 12 cm long. (Uniformity in width and texture of paper is of great importance in this comparison method. Irregular texture produces irregular impregnation with consequent inaccurate results.) To sensitize, soak strips in 3–6% (optimum 5%) filtered soln of  $\text{HgBr}_2$  in alcohol, 1 hour or longer according to quantity, character, and activity of Zn used. (Attenuated, unsatisfactory stains, due to over rapid evolution of arsine, can be shortened and intensified by increasing concentration of  $\text{HgBr}_2$  and vice versa.) If the strips are in sheets, cut off two sides before soaking and leave strips attached at ends. After sensitization remove strips and dry individual ones on glass rods, and groups by waving them in the air. Place strips when nearly dry between clean sheets of paper and subject them to pressure long enough to take out bends or curls. Store in dry dark place. (Aging of impregnated strips usually results in markedly fainter and longer stains. Desirable types of stain result from use of impregnated strips not over 2 days old.) When ready for use, cut individual strips off squarely half an inch from one end and insert this end into the narrow tube of apparatus. Handle sheets by the paper attached to either end and cut in half just before use. Strips must be clean and free of any contamination.

(g) *Standard arsenic soln.*—Dissolve 1 g of  $\text{As}_2\text{O}_3$  in 25 ml of 20% NaOH soln. Saturate soln with  $\text{CO}_2$  and dilute to 1 liter with recently boiled  $\text{H}_2\text{O}$ . 1 ml of this soln contains 1 mg of  $\text{As}_2\text{O}_3$ . Dilute 40 ml of this soln to 1 liter. Make 50 ml of the diluted soln to 1 liter and use to prepare standard stains. 1 ml of latter soln contains 0.002 mg of  $\text{As}_2\text{O}_3$ . A soln containing 0.001 mg of  $\text{As}_2\text{O}_3$  may also be prepared if desired. Prepare fresh dilute solns at frequent intervals.

## 29.2

## APPARATUS

(a) *Generators and absorption tubes.*—Use 2 oz. wide-mouth bottles of uniform capacity and design as generators, and fit each by means of perforated stopper with glass tube 1 cm in diam. and 6–7 cm long, with additional constricted end to facilitate connection. Place small wad of glass wool in constricted bottom end of tube and add 3.5–4 g of the 30-mesh cleaned sand, taking care to have same quantity in each tube. Moisten sand with 10% Pb acetate soln and remove excess by light suction. Clean sand when necessary by treatment (do not remove sand from tube) with  $\text{HNO}_3$  followed by  $\text{H}_2\text{O}$  rinse and suction. Treat with the Pb acetate soln. If sand has dried thru disuse, clean and remoisten it as directed. Connect tube by means of rubber stopper with narrow glass tube 2.6–2.7 mm in internal diam. and 10–12 cm long, and introduce the clean end of the strip of  $\text{HgBr}_2$  paper. (A 3 mm bore allows strip to curl, which results in an uneven stain and poor end point.) Clean and dry tube before inserting the  $\text{HgBr}_2$  paper. (An ordinary pipe cleaner may be used.)

(b) *Water bath.*—Use any constant temp. water bath. If no water bath is available, use any flat-bottomed container of suitable depth and capacity. (A deep water bath is suggested to insure uniform conditions during evolution and absorption of the As.)

## 29.3

## PREPARATION OF SAMPLE

(a) *For fresh fruits (apples, pears or similar products).*—Weigh and peel representative sample of fruit (1–5 lbs.). At blossom and stem ends cut out all flesh thought to be contaminated with As compounds and include with peelings. Place peelings in 1 or more 800 ml Pyrex Kjeldahl flasks. (As-free Pyrex glassware and “wet ashing” apparatus of Duriron are now available.) Add 25–50 ml of  $\text{HNO}_3$ , then add cautiously 20 ml of  $\text{H}_2\text{SO}_4$ . Place each flask on asbestos mat with 2" hole. Warm slightly and discontinue heating if foaming becomes excessive. When reaction has quieted, heat cautiously and rotate flask from time to time to prevent caking of sample upon glass exposed to flame. Maintain oxidizing conditions in flask at all times during digestion by adding cautiously small quantities of  $\text{HNO}_3$  whenever mixture turns brown or darkens. Continue digestion until organic matter is destroyed and  $\text{SO}_2$  fumes are copiously evolved. (Final soln should be water-white, or at most a light straw color.) Cool slightly and add 75 ml of  $\text{H}_2\text{O}$  and 25 ml of saturated  $\text{NH}_4$  oxalate soln to assist in expelling oxides of N from the soln. Evaporate again to point where fumes of  $\text{SO}_2$  appear in neck of flask. Cool, and dilute with  $\text{H}_2\text{O}$  to 500 or 1000 ml in volumetric flask.

(b) *For dried fruit products.*—Prepare sample by alternately grinding and mixing 4–5 times in food chopper. Place 35–70 g portions in 800 ml Kjeldahl flasks, and add 10–25 ml of  $\text{H}_2\text{O}$ , 25–50 ml of  $\text{HNO}_3$ , and 20 ml of  $\text{H}_2\text{SO}_4$ . Continue digestion as directed in (a). Dilute digested soln to 250 ml.

(c) *For small fruits, vegetables, etc.*—Use 70–140 g of sample and digest as directed under (a) and (b).

(d) *For materials other than (a), (b), or (c).*—Digest 5–50 g, according to degree

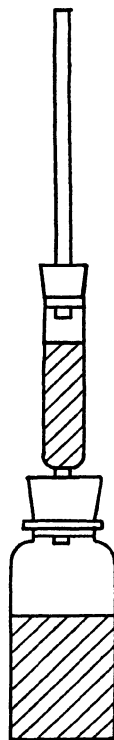


FIG. 44.—GENERATOR TO BE USED WITH GUT-ZEIT METHOD FOR DETERMINATION OF ARSENIC

of dryness and amount of As expected, as directed under (a) and (b). Dilute to definite volume dictated by circumstances.

(e) *For products containing stable organic As compounds, products liable to yield incompletely oxidized organic derivatives that inhibit arsine evolution, or products that are otherwise especially difficult to digest.*—Shrimp, tobacco, oils, and sometimes other products require special treatment to complete oxidation of organic As to inorganic  $As_2O_3$ , or to destroy organic interferences previous to As determination. For details consult following references:

- (1) C. R. Gross, *Ind. Eng. Chem., Anal. Ed.*, **5**, 58 (1933).
- (2) Carey, Blodgett, and Satterlee, *Ibid.*, **6**, 327 (1934).
- (3) Remington, Coulson, and von Kolnitz, *Ibid.*, 280.
- (4) C. C. Cassil, *J. Assoc. Official Agr. Chem.*, **20**, 171 (1937).

Dilute the As solns obtained by these special methods of preparation to definite volume.

(f) *For ultra-micro quantities of As, very labile forms of As, and a vacuum-accelerated Gutzeit reduction system for mercuric bromide spot filtration.*—Consult Satterlee and Blodgett, *Ind. Eng. Chem., Anal. Ed.*, **16**, 400 (1944).

## 29.4

### ISOLATION OF ARSENIC

Before making determinations isolate the As, when interfering substances are present in digests (pyridine from tobacco), or when samples contain excessive amounts of salts, or  $H_2SO_4$  from digestions. Consult Ref. (1), 29.3(e) for method of isolation of As after digestion, or isolate As by  $AsCl_3$  distillation as directed in bromate method, 29.9. Gelatin may be hydrolyzed with HCl and the As isolated as directed in Ref. (1), 29.3(e).

## 29.5

### DETERMINATION

Determine the acid (HCl or  $H_2SO_4$  according to previous treatment), by titration if necessary, in definite volume of sample soln. Place aliquots containing 0.01–0.03 mg of  $As_2O_3$  (0.020–0.025 mg is optimum), and not larger than 30 ml, in Gutzeit generators. If As in aliquot taken is found to be outside limits specified, repeat with proper aliquot. If aliquot contains only HCl, add sufficient HCl to make total volume of 5 ml; if it contains  $H_2SO_4$ , add sufficient 25% As-free NaOH soln (keep in As-free Pyrex) to exactly neutralize it and add 5 ml of HCl, or add sufficient HCl to the  $H_2SO_4$  in aliquot to make total volume of 5 ml. Cool when necessary and add 5 ml of the KI reagent and 4 drops of the  $SnCl_2$  soln, 29.1(a). Prepare standards corresponding to 0.010, 0.020, and 0.030 mg of  $As_2O_3$  from Reagent (g), 29.1. Since the standards *must* contain same kind and amounts of acid as samples, add 5 ml of HCl, or  $H_2SO_4$ , and HCl (total 5 ml) according to prior treatment of unknown. If the  $H_2SO_4$  has been neutralized, add an equivalent quantity of As-free  $Na_2SO_4$  to standards. Mix, and allow to stand for 30 min. at not less than 25° or 5 min. at 90°. Dilute with  $H_2O$  to 40 ml.

Prepare generator as directed under 29.2 and center strip of  $HgBr_2$  paper carefully in the narrow tube. According to activity of the Zn, add to each of standards and samples 10–15 g of activated stick Zn or 2–5 g of granulated Zn and add the same quantity to each generator. Equalize as far as possible surface area of Zn exposed in standard and sample. If sheets of strips are used, prepare sample and standard strips from same strip-group.

Immerse apparatus to within 1" of top of narrow tube in water bath, which is kept at constant temp. of 20–25°, and allow evolution to proceed for 1.5 hours. Remove strip and average length of stains on both sides in mm. Plot graph of

standard strips on cross-sectioned paper, using length in mm as ordinate and the mg of  $\text{As}_2\text{O}_3$  as abscissa. (Preparation of standard graph averages errors of individual standards. Reading strip from such a graph is considered more convenient and accurate than comparing strips themselves.) Locate length of unknown strip on standard graph and read off on abscissa quantity of As present. Report only to third decimal as grains of  $\text{As}_2\text{O}_3$ /lb. Take smaller or larger aliquots when stain is longer or shorter than highest or lowest standard, respectively. Grain/lb.  $\times 143$  = p.p.m.; p.p.m.  $\times 0.007$  = grain/lb.

Frequent blanks should be made. With reagents of suitable quality, blanks should not show more than 0.001 mg of  $\text{As}_2\text{O}_3$ .

#### Bromate Method (2)—Tentative

(Applicable to determination of As in plants and food products where a sample of convenient size for digestion will yield at least 0.005 grain (0.324 mg) of  $\text{As}_2\text{O}_3$ )

#### 29.6

##### REAGENTS

(a) *Ammonium oxalate-urea soln.*—To saturated  $\text{H}_2\text{O}$  soln of  $\text{NH}_4$  oxalate add 50 g of urea/liter.

(b) *Hydrazine sulfate-sodium bromide soln.*—Dissolve 20 g of hydrazine sulfate and 20 g of NaBr in 1 liter of HCl (1+4).

(c) *Sodium chloride.*—Commercial salt, uniodized.

(d) *Standard potassium bromate soln.*—Dissolve 0.1823 g of  $\text{KBrO}_3$  in  $\text{H}_2\text{O}$  and dilute to 1 liter. 1 ml = 0.005 grain of  $\text{As}_2\text{O}_3$ . Standardize by titration against the standard  $\text{As}_2\text{O}_3$  soln, (e), making titration at ca  $90^\circ$  and in presence of ca 100 ml of  $\text{H}_2\text{O}$  and 25 ml of HCl, in order to simulate conditions under which samples will be titrated. 1 ml of the  $\text{KBrO}_3$  soln should be equivalent to 1 ml of  $\text{As}_2\text{O}_3$  soln.

(e) *Standard arsenious oxide soln.*—Dissolve 0.3241 g of  $\text{As}_2\text{O}_3$  in 25 ml of 10% NaOH soln, make slightly acid with  $\text{H}_2\text{SO}_4$  (1+6), and dilute with  $\text{H}_2\text{O}$  to 1 liter.

#### 29.7

##### DISTILLING APPARATUS

The distilling apparatus consists of 800 ml Kjeldahl flask (A), distilling tube (B), and 300 ml Erlenmeyer flask (C).

To prepare distilling tube, bend 10–15 mm glass tube to acute angle of ca  $70^\circ$ . Draw the longer arm, which is ca 15–20" long, down to orifice of ca 3 mm. Fit shorter arm (ca 4") with No. 7 rubber stopper, which has previously been boiled in 10% NaOH soln for 15 min., and then in HCl for 15 min., in order to remove most of the S compounds which might be distilled and react with the  $\text{KBrO}_3$  soln. All glass apparatus (Ramberg-Sjöström apparatus, Fig. 67, 39.193, for example) is very useful and will reduce blanks to minimum.

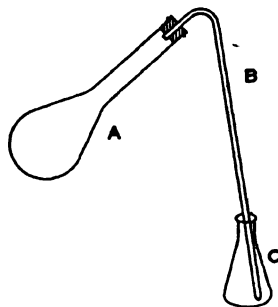


FIG. 45.—DISTILLING APPARATUS FOR DETERMINATION OF ARSENIC BY BROMATE METHOD

#### 29.8

##### PREPARATION OF SAMPLE

Introduce a suitable sample containing 0.005 grain (0.324 mg) or more of  $\text{As}_2\text{O}_3$  into 800 ml Kjeldahl flask. Proceed with acid digestion as directed under 29.3, with following exception: Add exactly 20 ml of  $\text{H}_2\text{SO}_4$ , or (rarely), if material is difficult

to digest, exactly 25 ml, at beginning of digestion. After digestion is complete, add 50 ml of  $\text{H}_2\text{O}$  and 25 ml of the  $\text{NH}_4$  oxalate-urea soln, and boil until white  $\text{SO}_2$  fumes extend up into neck of flask to decompose oxalates and urea completely. (Volatile intermediate products may titrate with  $\text{KBrO}_3$ . If heat available is insufficient to decompose these substances, it is preferable to evaporate to fumes with  $\text{H}_2\text{O}$  alone. Hydrazine sulfate will destroy small amounts of oxides of N.)

## 29.9

## ISOLATION

Add 25 ml of  $\text{H}_2\text{O}$  to digested soln in the Kjeldahl flask and cool to room temp. Put 100 ml of  $\text{H}_2\text{O}$  into flask C. Add to soln in Kjeldahl flask 20 g of  $\text{NaCl}$  and 25 ml of the hydrazine sulfate- $\text{NaBr}$  soln and connect distilling tube. Heat Kjeldahl flask over small well-protected flame, and distil into  $\text{H}_2\text{O}$  in Erlenmeyer flask. (Heating is not intended to boil soln but to bring about evolution of  $\text{HCl}$  gas, which carries over the  $\text{AsCl}_3$  with it. Absorption of evolved  $\text{HCl}$  gas by  $\text{H}_2\text{O}$  causes rise in temp., which indicates progress of distillation.) Adjust flame so that temp. of distillate soln will rise to  $90^\circ$  in 9–11 min. and then discontinue distillation. (Residual mixture in flask should not be less than 55 ml.) If distillation proceeds further, or larger quantity of  $\text{H}_2\text{SO}_4$  than that specified is used in the digestion,  $\text{SO}_2$  is distilled, which is titrated as As.

## 29.10

## DETERMINATION

Titrate distillate at once with the  $\text{KBrO}_3$  soln, using 3 drops of methyl orange indicator. (Single drops of indicator, 6.3(f), but not exceeding 3, may be added during titration as red color fades.) Towards end of titration add the  $\text{KBrO}_3$  soln very slowly and with constant agitation to prevent local excess. End point is reached when a single drop of the  $\text{KBrO}_3$  just destroys the final tinge of red color. Use Erlenmeyer flask containing clear  $\text{H}_2\text{O}$  for comparison. (End point must not be exceeded as action of indicator is not reversible and back titrations are not reliable. At proper end point, red color produced by 2 additional drops of methyl orange indicator should persist for at least 1 min.) Correct results for volume of  $\text{KBrO}_3$  used in blank run (digest 5 g of pure sucrose) with same reagents (same quantities) and regular distillation procedure. (Blank titration should not exceed 0.7 ml of  $\text{KBrO}_3$  soln. The method is accurate down to variations in blank, which should not exceed 0.1 ml when chemicals from same lot are used.) Should blank titration be high or variable, test individual reagents for purity by  $\text{KBrO}_3$  titration and discard unsatisfactory ones. Test the  $\text{H}_2\text{SO}_4$  by bringing 20 ml to boil, cooling, diluting with  $\text{H}_2\text{O}$  to 100 ml, adding a little  $\text{HCl}$ , and titrating while hot. (It probably will furnish most of the blank.) Select rubber stoppers carefully as they are often the source of unsatisfactory blanks.

If high results, due to  $\text{SO}_2$  produced during distillation, or other reducing substances, are suspected, dilute titrated distillate to definite volume and redetermine the As in aliquot by Gutzeit method, 29.1–29.5. Positive test for sulfates in aliquot of titrated distillate indicates contamination with reduced S compounds and a necessity for check on the As.

## COPPER

*Volumetric Method (3)—Tentative*

(Minimum of 1 mg Cu)

## 29.11

## PREPARATION OF SAMPLE

Digest 50–100 g sample as directed under 29.3, or ash it as described under 29.37.

## 29.12

## REAGENTS

(a) *Standard copper soln.*—Dissolve 318 mg of pure metallic Cu in  $\text{HNO}_3$  and evaporate to dryness on steam bath. Add sufficient  $\text{H}_2\text{O}$  and a few drops of acetic acid to dissolve the  $\text{Cu}(\text{NO}_3)_2$  and again evaporate to dryness on steam bath. Redissolve the  $\text{Cu}(\text{NO}_3)_2$  as above and make up to 1 liter.

(b) *Sodium thiosulfate soln.*—Dissolve 24.82 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in 1 liter of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  to make an approximate 0.1 *N* soln. Allow to stand, preferably ca 2 weeks. Prepare 0.005 or 0.01 *N* solns by dilution of this reagent with  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  in the ratio of 1:20 or 1:10. Standardize daily against the standard Cu soln in following manner: Place 20 ml of the standard Cu soln in 100 ml Erlenmeyer flask, add excess of  $\text{NH}_4\text{OH}$ , and continue as directed under 29.13, beginning "and boil gently to drive off excess  $\text{NH}_3$ ." 1 ml of 0.01 *N*  $\text{Na}_2\text{S}_2\text{O}_3$  = 0.6357 mg of Cu.

## 29.13

## DETERMINATION

Dissolve ashed sample in HCl and neutralize this soln, or neutralize the soln obtained by the wet digestion, with  $\text{NH}_4\text{OH}$ . Add 5 ml of  $\text{H}_2\text{SO}_4$ , dilute soln to 200 ml, and boil for 1 min. Add cautiously 10 ml of hot saturated  $\text{Na}_2\text{S}_2\text{O}_3$  soln and continue boiling for 5 min. (With larger quantities of Cu the precipitate coagulates, and the liquid becomes practically clear. A few ml of 1%  $(\text{NH}_4)_2\text{SO}_4$  soln may be added to hasten coagulation.) Filter precipitate and wash 6 times with hot  $\text{H}_2\text{O}$ . Reserve filtrate for determination of Zn, if necessary. Fold precipitate within filter paper, place in small crucible, and ignite in electric muffle at  $500^\circ$ . Treat residue with 1 ml of  $\text{HNO}_3$  (2+5) and dry on steam bath. Add 5 ml of  $\text{H}_2\text{O}$  and again evaporate to dryness on steam bath. Add 20 ml of  $\text{H}_2\text{O}$  and an excess of  $\text{NH}_4\text{OH}$  and heat on steam bath until Cu salts are dissolved. Transfer to 100 ml Erlenmeyer flask and boil gently to drive off excess  $\text{NH}_3$ . Make acid to litmus paper with acetic acid (1+1), add 1 ml in excess, boil soln 1 min., and cool to room temp. Add 2 g of KI dissolved in enough  $\text{H}_2\text{O}$  to make final soln 50 ml, and titrate the free I immediately with 0.01 *N* or 0.005 *N*  $\text{Na}_2\text{S}_2\text{O}_3$  (according to amount of Cu present, as shown by degree of blue color in ammoniacal soln) until end point is nearly reached. Add 2 ml of starch soln, 29.36(h), and continue titration dropwise to disappearance of blue color. Compare with titrated standard.

*Diethyldithiocarbamate Method (29)—Tentative*

## 29.14

## PRINCIPLES

The general method calls for sample preparation by wet combustion, isolation of Cu as the dithizone complex by extraction from acid solution containing KI, stripping from dithizone solution, and final colorimetric estimation as diethyldithiocarbamate. In a special short method applicable under restricted conditions, preliminary isolation of Cu is omitted. The preliminary extraction with dithizone separates Cu from common heavy metals including Bi, Co, and Ni, which would interfere in the carbamate determination unless removed. Bi, Co, and Ni must be absent when the short carbamate method is used. The short method is unsuitable when large amounts of alkaline earth phosphates are present, although if amount of Cu present is relatively large this handicap may sometimes be overcome by reducing size of sample. Instructions apply to ranges from 0–10 to 50–250 micrograms of Cu. In the complete method, quantity of Cu should be greater than 10 micrograms to minimize effect of variations in blank.

## 29.15

## PRECAUTIONS

Clean glassware with hot  $\text{HNO}_3$ . Grease lower stopcocks of separators with white petrolatum and do not use brass chains. Purify  $\text{HNO}_3$  and  $\text{NH}_4\text{OH}$  by distillation in Pyrex, and other reagents as directed below. Use  $\text{H}_2\text{O}$  redistilled from Pyrex for all dilutions and in reagents not specially purified.

Discarded  $\text{CCl}_4$  may be recovered as directed by Biddle (4) and Clifford (5).

## 29.16

## REAGENTS

(a) *Diphenylthiocarbazone (dithizone)*.—Purify as directed in 29.36(e) and make soln 25 mg/liter in purified  $\text{CCl}_4$ . Store in refrigerator.

(b) *Potassium iodide soln.*—10%. Dissolve 50 g of KI and make up to 500 ml. Place in separator with  $\text{CHCl}_3$  soln of dithizone. Add  $\text{NH}_4\text{OH}$  dropwise with shaking until dithizone begins to transfer to aqueous phase. Extract with further portions of dithizone until extracts are green, then add HCl dropwise until dithizone is precipitated from aqueous phase. Extract with  $\text{CCl}_4$  and filter. If free I appears on standing, discharge with dilute  $\text{Na}_2\text{S}_2\text{O}_3$  or  $\text{Na}_2\text{SO}_3$  soln added dropwise.

(c) *Potassium iodide soln.*—2%. To 100 ml of the 10% KI soln add 5 ml of 1 N HCl or equivalent and dilute to 500 ml. If free I appears on standing, decolorize as directed for (b).

(d) *Ammonium citrate soln.*—Dissolve 150 g of citric acid in 600 ml of  $\text{H}_2\text{O}$  and make just alkaline to cresol red (alkaline range) with  $\text{NH}_4\text{OH}$ , using spot plate; add 10 ml of  $\text{NH}_4\text{OH}$  in excess and dilute to 1 liter. Extract with dithizone soln in  $\text{CHCl}_3$  until extracts are green, then with  $\text{CHCl}_3$  until extracts are colorless, and finally with  $\text{CCl}_4$ . Filter.

(e) *Sodium diethyldithiocarbamate soln (carbamate soln.)*.—Dissolve 1 g of the salt in  $\text{H}_2\text{O}$ , dilute to 100 ml, and filter. Store in refrigerator.

(f) *Copper standard.*—Weigh accurately 0.2000 g of C.P. Cu wire or foil and place in 125 ml Erlenmeyer flask. Add 15 ml of  $\text{HNO}_3$  (1+4), cover with a watch-glass, and allow to dissolve, warming to complete soln. Boil to expel fumes, cool, and dilute to 200 ml. Prepare intermediate standard by diluting 20 ml of this soln to 200 ml. This intermediate standard contains 0.1 mg Cu/ml. The working standard, prepared just before use, consists of either 50, 20, or 10 ml of the intermediate standard made up to 1 liter, and contains 5, 2, or 1 micrograms of Cu/ml.

(g) *Mercuric chloride stripping soln.*—To 10 ml of 0.05 M  $\text{HgCl}_2$  soln (1.36 g/100 ml) add 4 ml of HCl and dilute to 1 liter.

(h) *Sodium sulfate soln.*—10%. Dissolve 50 g of anhydrous  $\text{Na}_2\text{SO}_4$  and 2 g of  $\text{Al}_2(\text{SO}_4)_3 \cdot \text{K}_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$  in 400 ml of  $\text{H}_2\text{O}$ , add 2 ml of  $\text{NH}_4\text{OH}$ , and mix. Add 5 ml of the carbamate soln, make to 500 ml, mix, and filter.

## 29.17

## PREPARATION OF SAMPLE

Quantity of material taken depends upon range of instrument used for final estimation and upon whether final determination is to be made on entire sample or an aliquot. If sample is prepared by micro wet combustion, entire sample may be used, and size should then be such that expected total amount of Cu will fall within useful range of instrument used. When macro wet combustion is employed, sample should furnish 10 or 20 times the amount of Cu needed for estimation. In general, proceed as directed under 29.3, but as soon as digest remains yellow and does not char on evolution of  $\text{SO}_2$  fumes, add 60–70%  $\text{HClO}_4$  in volume  $\frac{1}{2}$  to  $\frac{1}{4}$  of that of the  $\text{H}_2\text{SO}_4$  and continue heating until all  $\text{HClO}_4$  is expelled. Cool, and if macro wet combustion is used, dilute to 200 ml in volumetric flask. If micro diges-

tion apparatus is used, reduce size of sample and quantity of acids correspondingly and dilute with 10–20 ml of  $H_2O$  before transfer.

Run blanks with all samples. Since  $H_2SO_4$  is the least controllable source of reagent blank, the amount should be as small as practicable in proportion to size of sample.

### GENERAL METHOD

#### 29.18

#### ISOLATION OF COPPER

In 250 ml short-stemmed separator place 10 ml of the ammonium citrate soln and add the sample soln or aliquot. Dilute to ca 85 ml, add 0.5 ml of bromophenol blue indicator and  $NH_4OH$  dropwise until bluish tinge of indicator appears, then add 2 *N*  $HCl$  dropwise until bluish tinge changes to yellow (pH 3.0–3.3), avoiding any excess of acid. Adjustment of pH may be facilitated by comparison with series of phthalate buffers, pH 3.0–4.0, or by reference to a color chart for the indicator, but these are usually unnecessary. Add 10 ml of the 10%  $KI$  soln and extract with 10 ml of dithizone soln in  $CCl_4$ , 25 mg/liter, shaking vigorously for 2–3 min. Repeat extraction with successive 10 ml portions of dithizone until color after shaking indicates definite excess of dithizone, then with one additional portion, which should remain green. Combine extracts in second separator containing 25 ml of the 2%  $KI$  soln. Discard sample soln, and wash and drain first separator. Shake combined extracts with the 2%  $KI$  soln for 30 seconds and draw off extract into first separator. Shake the 2%  $KI$  soln 2 min. with 10 ml of the dithizone soln, add this to the extract in the first separator, and discard the  $KI$  soln. To extracts in first separator add 20 ml of the  $HgCl_2$  stripping soln and shake vigorously for 1 min. to strip  $Cu$  from the dithizone layer. Allow to separate, and draw off and discard the  $CCl_4$ . Rinse the aqueous layer with two 5 ml portions of  $CCl_4$ . Add 2 drops 0.02% cresol red soln and  $NH_4OH$  dropwise to the purplish red color of the indicator, then add 5 ml of the  $Na_2SO_4$  soln and 1 ml of the carbamate soln. Extract the  $Cu$  with two 10 ml portions of  $CCl_4$ , shaking vigorously for 1 min. with each portion. Combine the extracts in 50 ml Erlenmeyer flask, add a glass bead, and evaporate to dryness on water bath or hot plate. (Evaporation may be hastened by gentle stream of filtered air.) When  $CCl_4$  is expelled, add 1 ml of  $H_2SO_4$  and 1 ml of 60–70%  $HClO_4$ , and heat on hot plate until  $HClO_4$  is expelled, regulating temp. to avoid spattering. Cool, and add 10 ml of  $H_2O$  and 2 drops of 0.02% cresol red soln, then  $NH_4OH$  to the purplish red color of the indicator. Cool, and transfer to separator, using enough  $H_2O$  to give volume of 50 ml, and proceed as directed under 29.19.

#### 29.19

#### DETERMINATION

To the soln obtained in 29.18 add 1 ml of the carbamate soln and exactly 10 (or 20) ml of  $CCl_4$  or  $C_6H_5Br$ . Shake 2 min. and allow layers to separate. Draw off lower layer thru pledget of cotton in stem of small funnel into test tube or small flask. If  $CCl_4$  is used, stopper until examination can be made. Determine absorption, transmission, or scale reading in suitable photometer or photoelectric colorimeter, utilizing monochromatic or nearly monochromatic light at central wave length of ca 440  $m\mu$ , and calibrated in suitable range, or compare with standards in plunger-type colorimeter. Latter method is less sensitive and accurate than photometric measurement; in using it prepare standards as nearly as practicable of same concentration as unknowns in order to minimize errors due to departure from Beer's law. For neutral wedge photometer and photoelectric colorimeters, useful range is from 0–10 to 0–200 micrograms of  $Cu$ , depending on type of instrument, size of cell, and volume of soln; for plunger type colorimeter it is 50–250 micrograms. For deter-



mination with neutral wedge photometer use the following table of volumes and cell lengths:

<i>Cu range</i> <i>micrograms</i>	<i>Volume</i> <i>ml</i>	<i>Cell length</i> <i>inches</i>
0-10	10	$\frac{4}{2}$
0-25	10	2
0-50	10	1
0-100	10	$\frac{1}{2}$
0-200	20	$\frac{1}{4}$

**29.20****PREPARATION OF STANDARDS AND CALIBRATION CURVES**

For calibration of photometric instruments prepare a series of standards including zero Cu and five steps in the selected range. For plunger-type colorimeter prepare at least three standards covering range of expected values.

Transfer requisite volume of the standard Cu soln to separator, adding H<sub>2</sub>O to total volume of 40 ml. Add 2 drops 0.02% cresol red soln and NH<sub>4</sub>OH to purplish red color of indicator, then 10 ml of the Na<sub>2</sub>SO<sub>4</sub> soln, and proceed as directed under 29.19, beginning "add 1 ml of the carbamate soln."

If photometric readings are in terms of optical density or proportional units, plot readings against quantity of Cu on ordinary graph paper. If readings are in per cent transmission, use semilog paper, plotting transmission on the logarithmic scale and quantity of Cu on the linear scale. There is usually some deviation from linearity, and values for unknowns are best read from smooth curve approximating the standard points.

**29.21****SHORT METHOD**

(Bi, Co, and Ni absent. Not applicable to large samples of materials high in alkaline earth phosphates.)

To the sample soln or suitable aliquot in separator, add 10 ml of the ammonium citrate soln and 2 drops of 0.02% cresol red soln. Add NH<sub>4</sub>OH dropwise to purplish red color of indicator. Cool, and dilute to 50 ml. Proceed as directed under 29.19, beginning "add 1 ml of the carbamate soln."

**FLUORINE—TENTATIVE****29.22****PRINCIPLES**

The general method specifies an ashing treatment of the sample with Ca(OH)<sub>2</sub> as F fixative, isolation of F by means of a Willard-Winter distillation (6) from HClO<sub>4</sub>, and estimation in distillate by a Th(NO<sub>3</sub>)<sub>4</sub> "back-titration" procedure (7). The technique and reagent strengths are designed to handle conveniently not more than 10.0 mg of F. Modifications of this general procedure, applicable to specific products, are described.

**29.23****PRECAUTIONS AND INTERFERENCES**

Control, by means of careful choice and purification of reagents, the magnitude of a determination blank (*see* 29.25). With care the blank will be low (1-3 micrograms of F), but with low F foods it may represent a considerable part of the total F determined. Hence it must be stable. A large part of it will be a "distillation blank" apparently resulting from F leached from the glassware of the still during distillation. This blank can be minimized by preliminary treatment of still, 29.27, and it should be possible to deduce and correct for an average distillation blank if

still of same material and design are routinely used; otherwise, each still must bear its special blank. New, unused stills will usually be found to exhibit a high blank, which will diminish to constant low figure after several runs. They should not be used until the analyst has assured himself, by means of several consecutive blank runs, that the still is yielding not more than a constant, low amount of F. Check ashing utensils by blank runs with the fixative soln to ascertain whether or not they contribute appreciable F. Even Pt vessels may become contaminated (owing presumably to slight Ca content) if they have been used recently for HF volatilization of  $\text{SiO}_2$ . In addition, such blank runs are useful for testing the reagents and apparatus used in the method and also the evaporators, hoods, muffles, and laboratory atmosphere for presence of F fumes and dust. If HF bottles are permitted in same laboratory, seal them immediately after use, and avoid contamination from roach powders. Ordinary tap  $\text{H}_2\text{O}$  may be a source of F contamination, since 1 ml of a water containing 2 p.p.m. of F will contribute 2 micrograms of F if allowed to remain or to dry in a still. For this reason routinely rinse all glassware (stills, flasks, burets, etc.) with  $\text{H}_2\text{O}$ , preferably redistilled from alkaline  $\text{KMnO}_4$ . Filter papers may contribute small (microgram) quantities of F, and glass filters are preferred should a filtration be required in the microdetermination.

*Interferences* are gelatinous  $\text{SiO}_2$ , Al, and B compounds, which repress the evolution of F as  $\text{H}_2\text{SiF}_6$  in the distillation; materials such as nitrates, nitrites, peroxides, Cl,  $\text{SO}_2$ , and  $\text{H}_2\text{S}$ , which act upon the indicator in the titration or otherwise interfere; halides (chloride), which distil to give excessive acidity in the distillate; and phosphates and sulfates, which react with Th in the titration to give high results. The procedure is so designed that most of these interferences are automatically eliminated, but the analyst should be on guard against their possible occurrence under unusual circumstances.

## GENERAL METHOD

### 29.24

#### APPARATUS

(a) *Fluorine still*.—A Claisen-type distilling flask of 100–125 ml size is most practical for general work. It must be of Pyrex glass, and auxiliary neck should be sealed off immediately above side-arm to prevent pocketing and refluxing of distillate. Still should be as small and simply designed as practicable, in fact ordinary distilling flasks can be used for some work and they are slightly more efficient than the Claisen type, except that there is more danger of spraying over of distilling acid. The still is equipped with a dropping funnel and 0–150° thermometer, the latter extending to within  $\frac{1}{4}$ " of bottom of flask, so that bulb is immersed in the boiling acid mixture. Acid-alkali washed beads, preferably of Pyrex, should be on hand. Rubber stoppers should previously be cleaned by boiling in 10% NaOH soln. All-glass apparatus with interchangeable accessories is convenient, especially in routine work, and eliminates need for rubber stoppers. While not entirely necessary for heating still, the use of a Wood's metal bath, adequately shielded, will prevent undue decomposition of  $\text{HClO}_4$  and aid materially in securing a low blank and a low-acid distillate; hence its use is strongly urged. If metal bath is used, take care that flask is not immersed so deeply that bath level is above that of liquid in flask; if the bath is not used, transite or asbestos shielding boards are essential, and flask should be heated thru small hole in such a shield by low "clean" flame. (The purpose of the bath and shielding boards is to prevent over-heating of the upper still walls.) At the analyst's option, distilling  $\text{H}_2\text{O}$  may be added as steam instead of thru the dropping funnel, and an electric boiler (8) is a convenient steam generator. If steam is used, inlet tube should dip below surface of liquid in still. One advantage in

adding the distilling  $H_2O$  thru a funnel is that last portions of rinse  $H_2O$  used in transferring an ash can be used in the distillation. If the funnel plug is thinly notched with sharp file on either side of the bore, dropping rate can be more easily controlled, and end of the funnel stem need not extend into liquid in still. The still is used in conjunction with clean straight-tube condenser no longer than necessary for adequate cooling. (Vertical arrangement of condenser will conserve bench space.)

(b) *Nessler tubes*.—Tall form, 100 and 50 ml sizes, glass-stoppered type preferred. Matched in sets of at least six. (The 100 ml size will be used more frequently in the general procedure.)

(c) *Additional apparatus*.—In addition there will be required (see 29.23) carefully cleaned and tested Pt, or well-glazed porcelain, dishes of at least 100 ml size; 150 ml volumetric flasks, or if these are not available, the 200 ml size; and 10 ml burets (conveniently automatic) to deliver the various solns required in distillation and titration. An overhead radiant heater will be found invaluable for the drying and preliminary charring of samples, especially those of the high sugar type.

## 29.25

### REAGENTS

(a) *Lime suspension*.—Carefully slake ca' 56 g (1 mol) of low F CaO (ca 2 p.p.m. of F) with ca 250 ml of  $H_2O$  and add 250 ml of 60%  $HClO_4$  slowly and with stirring. Add a few glass beads and boil down to copious fumes of acid; then cool, add 200 ml of  $H_2O$ , and boil down again. Repeat dilution and boiling down once more; cool, dilute considerably, and filter thru fritted-glass filter if precipitate of  $SiO_2$  has appeared. Pour the clear soln, with stirring, into 1 liter of 10% W/V NaOH soln, allow precipitate to settle, and siphon off supernatant liquid. Remove Na salts from precipitate by washing 5 times in large centrifuge bottles, shaking up substrate thoroly each time. Finally, shake precipitate into suspension and make to 2 liters. Preserve in paraffined bottles. (100 ml of this suspension should give no appreciable F blank when evaporated, distilled, and carried thru titration procedure described below.) Always shake suspension well before using.

(b) *Perchloric acid soln*.—60%. Dilute C.P.  $HClO_4$  with 3–4 volumes of  $H_2O$  and boil down to original volume. Do not fume strongly. Repeat, and preserve in Pyrex. (The prepared acid should give no test for chloride.)

(c) *Sulfuric acid soln*.—Carefully mix equal volumes of C.P.  $H_2SO_4$  and  $H_2O$ , boil down to fumes, cool, dilute carefully, boil down once more, and dilute to 1+1 volume.

(d) *Silver perchlorate soln*.—50% W/V.

(e) *p-Nitrophenol indicator*.—0.5% W/V alcoholic soln.

(f) *Potassium hydroxide soln*.—Exactly 0.05 N.

(g) *Potassium chloride soln*.—0.05 N. 3.727 g/liter.

(h) *Hydroxylamine hydrochloride soln*.—1.0% W/V.

(i) *Hydrochloric acid soln*.—Exactly 0.05 N.

(j) *Alizarin indicator*.—0.01% soln of sodium alizarin sulfonate (Alizarin Red S).

(k) *Potassium fluosilicate*.—If pure  $K_2SiF_6$  is not obtainable, prepare as follows: Introduce, thru dropping funnel, a saturated soln of NaF, or a suspension of crude  $K_2SiF_6$ , into 500 ml Claisen distilling apparatus containing 60 ml of 1+1  $H_2SO_4$ , some glass beads, and 10–20 g of powdered  $SiO_2$  (or glass) maintained at boiling temp. of 120–125°. Conduct distillate into soln of ca 25 g of pure KCl in  $H_2O$ , held at simmering temp. on hot plate so that distillate volumes do not become excessive. If necessary, add more  $H_2O$  to mixture by means of dropping funnel placed in side-neck of still. Regulate rate of addition of fluoride to still and temp. of condens-

ing  $\text{H}_2\text{O}$  so that side-arm and condenser do not become clogged with the evolved  $\text{H}_2\text{SiF}_6$ , which tends to lodge as a gelatinous mass.  $\text{K}_2\text{SiF}_6$  is formed in receiver and altho entirely crystalline it assumes appearance of a gelatinous substrate owing to small difference between its refractive index and that of aqueous soln. When a substantial amount has been collected, pour contents of receiver into large centrifuge bottle and wash repeatedly by means of centrifuge (shaking up precipitate thoroly each time), until washings are Cl free by test. Collect on Büchner funnel and either allow to air-dry or bring to constant weight in vacuo at  $50\text{--}70^\circ$ . Determine purity by means of a Travers titration (9) at boiling temp. with  $0.2\text{ }N\text{ NaOH}$  ( $1\text{ ml} = .01101\text{ g of K}_2\text{SiF}_6$ ); also by conversion to  $\text{K}_2\text{SO}_4$  by treating  $0.3\text{--}0.4\text{ g}$  in deep Pt dish with a little  $\text{H}_2\text{O}$ , then  $\text{H}_2\text{SO}_4$  plus a little  $\text{HF}$ , fuming off the excess acid *carefully* (if overheated, mixture has tendency to spatter), and heating to constant weight of  $\text{K}_2\text{SO}_4$  at  $650^\circ$ . With glass apparatus an entirely pure product is not usually obtained, as some contamination with  $\text{SiO}_2$  results from leaching effect of vapors on condenser. A pure product can be obtained by use of a Pt still.  $0.9660\text{ g}$  of pure  $\text{K}_2\text{SiF}_6$  made to 1 liter gives a stock soln containing  $0.5\text{ mg of F/ml}$ . Much more will not dissolve. Prepare such a soln, correcting this weight of  $0.9660$  by purity factor of the  $\text{K}_2\text{SiF}_6$  (figure for purity obtained from average of two above methods of assay). Preserved in paraffined bottle, soln will keep indefinitely.

Prepare the soln *used in the titration*, 29.28, by diluting  $20\text{ ml}$  of this stock soln to 1 liter ( $1\text{ ml} = 10\text{ micrograms of F}$ ). It will retain its strength for several weeks in ordinary volumetric ware.

(1) *Thorium nitrate soln.*— $0.25\text{ g of Th(NO}_3)_4 \cdot 12\text{H}_2\text{O}$  or  $0.20\text{ g of Th(NO}_3)_3 \cdot 4\text{H}_2\text{O}$  /liter.

Check the titer of the Th soln against the standard ( $10\text{ microgram/ml}$ ) F soln as follows: Measure  $10, 20, 30$ , etc., up to  $80\text{ micrograms of F}$  into  $100\text{ ml}$  Nessler tubes, and add  $4.00\text{ ml}$  of the  $0.05\text{ }N\text{ HCl}$  ( $2.00\text{ ml}$  if  $50\text{ ml}$  Nessler tubes are used, and carrying the range to only  $50\text{ micrograms of F}$  for this size tube) (10). Dilute mixture to ca the  $80$  (or  $40$ )  $\text{ml}$  mark and add  $1.00\text{ ml}$  of the  $1.0\%$   $\text{NH}_4\text{OH} \cdot \text{HCl}$  soln. Mix, then add exactly  $2.00\text{ ml}$  of the alizarin indicator (or  $1.00\text{ ml}$  for the smaller tube) and measure in the Th soln from a buret, mixing frequently until, when sighting down tube towards a white reflecting surface, an incipient pink or salmon pink color is observed. Add a little  $\text{H}_2\text{O}$  from time to time so that soln is nearly to mark as end point is approached. Finally, make exactly to mark and mix thoroly before checking final end point. Do not shake the tubes violently ( $5\text{--}6$  gentle inversions are sufficient). Make an effort to secure end-point shade intermediate between yellowish green of acid indicator and reddish purple of fully developed Th lake. Carry thru with series and plot  $\text{ml of Th soln}$  against  $\text{ml of standard fluoride}$  to obtain rough equivalence curve for the two solns. Depending upon quantity of F known to be present, add the Th soln in  $1\text{--}2\text{ ml}$  portions at first, with final additions of  $0.25\text{ ml}$

## 29.26

### PREPARATION OF SAMPLE

Methods of sample preparation are designed to furnish a representative sample in a workable quantity of material and to get sample in condition for final distillation. Mineralization by an ashing procedure is usually involved. Some mineral food products can be dissolved in and distilled from  $\text{HClO}_4$ , 29.27, provided no interferences appear in final distillate. In general,  $20\text{ g}$  or upwards of dry material,  $50\text{--}100\text{ ml}$  of liquid samples, and  $50\text{--}100\text{ g}$  of undried food products or plant material can be taken for analysis, depending upon expected F content, and the interferences.

such as excessive Cl, which use of large samples may introduce. For reasonable precision in analysis of low F foods the sample should be of sufficient size to yield titer of at least 0.5 ml for aliquot taken in final titration. However, it may not always be possible to handle this quantity of material. If adequate grinding and mixing equipment is available, it is often feasible to prepare large quantities of material (vegetables, mixed foods) and to take aliquant portions for analysis (11). Dry plant materials, feeds, bonemeal, etc., can be ground to convenient size in a Wiley mill and thoroly mixed before sample is taken. The following special methods for certain products are indicated:

(a) *Direct ashing*.—(Applicable to fibrous (not highly fatty) food materials, liquid samples, and in general to all foods that can be thoroly wet with an aqueous fixative soln. This procedure will apply to majority of food products.) Weigh suitable portion of prepared sample into clean Pt dish and add 25 ml of the  $\text{Ca}(\text{OH})_2$  suspension. (Porcelain casseroles or dishes are second choice because they may contribute small quantities of F, and  $\text{Al}_2\text{O}_3$ , to sample.) Mix in the  $\text{Ca}(\text{OH})_2$  suspension with glass rod, adding additional  $\text{H}_2\text{O}$  if necessary; rinse and remove rod. Dry *thoroly* on steam bath or in hot air oven, then slowly char sample by heating over low flame or electric stove with heat control. An overhead radiant heater is very convenient for both drying and charring sample. Control excessive swelling of high sugar foods by playing small flame over surface of sample from time to time, and char these products *slowly* so that excessive acidity is not generated. When sample is charred past danger of catching fire, place in muffle and ash at  $600^\circ$ . (For very small samples and minimum blanks it may be advisable to cover ashing vessel with inverted Pyrex Petri dish while ashing.) When a clean ash is obtained, cool dish and wet ash with ca 10 ml of  $\text{H}_2\text{O}$ . (Small amount of unburned C does not interfere, but if much is apparent, dry down and repeat ashing.) Cover dish with watch-glass and cautiously introduce under cover an amount of the  $\text{HClO}_4$  just sufficient to dissolve the ash. Rinse down cover with a little  $\text{H}_2\text{O}$  and transfer soln to the freshly prepared F still (29.27) thru long-stemmed funnel. Rinse dish with remainder of distilling acid, using ca 20 ml in all, and adding and transferring in several small portions. *Do not prolong transferring operation*. Finally rinse funnel and stirring rod into dish, assemble still, and complete rinsing of dish with several small portions of  $\text{H}_2\text{O}$ , pouring these into dropping funnel of still. If distilling  $\text{H}_2\text{O}$  is added as steam, 29.24(a), rinse dish with a little additional  $\text{H}_2\text{O}$  and add directly to acid mixture in still, but avoid excessive initial volume. Add ca 6 Pyrex beads and sufficient  $\text{AgClO}_4$  soln to precipitate all chloride. (Excess  $\text{AgClO}_4$  in reasonable quantity does no harm, and sufficient solid  $\text{Ag}_2\text{SO}_4$  may also be used.) Proceed as directed in 29.27.

(b) *Preliminary distillation*.—(Necessary with certain products of high phosphate content, such as Ca phosphate and bone meal, in order to eliminate the distilled  $\text{H}_3\text{PO}_4$  that may be present in appreciable quantities in first distillates. Also advisable with certain excessively fatty materials that may not be thoroly wet with the  $\text{Ca}(\text{OH})_2$  fixative, thus causing F loss in a direct ashing procedure.)

(1) *For inorganic phosphatic materials*, such as Ca phosphate, weigh sample, usually 10 g, into still, add a few glass beads, sufficient  $\text{AgClO}_4$  to precipitate possible Cl, and ca 20 ml of the  $\text{HClO}_4$ . If the inorganic phosphatic material does not contain excessive Ca, use similar quantity of the 1+1  $\text{H}_2\text{SO}_4$ . Distil at  $135\text{--}140^\circ$ , collecting ca 200 ml of distillate. (For this preliminary distillation, extreme care in securing a low-acid distillate is not essential.) Evaporate distillate to dryness in Pt after addition of excess of the  $\text{Ca}(\text{OH})_2$  suspension, assuring alkaline conditions by testing with drop of phenolphthalein indicator. (If  $\text{H}_2\text{SO}_4$  is used in this preliminary distillation, add to distillate a few drops of *F-free* 30%  $\text{H}_2\text{O}_2$  to remove possible sulfites.)

Heat dried residue at 600° for a few minutes to destroy indicator residues and possible  $\text{Cl}_3$ -containing compounds. Transfer contents of dish to freshly prepared still (29.27) with the 20 ml of distilling  $\text{HClO}_4$  as directed in (a), and proceed with final distillation as directed in 29.27.

Take 20 ml samples of sirupy  $\text{H}_3\text{PO}_4$  and collect at least 300 ml of first distillate at 135°, allowing the  $\text{H}_3\text{PO}_4$  to function as its own distilling acid. (More distillate is necessary because the  $\text{H}_3\text{PO}_4$  is less effective as a F distilling acid.) Neutralize with the  $\text{Ca}(\text{OH})_2$  suspension, evaporate to dryness, transfer to prepared still as directed above, and proceed as directed in 29.27.

(2) *For organic phosphatic materials*, such as bone meal, feed supplements, etc., give sample preliminary ashing treatment to destroy most of organic matter. For this purpose, moisten sample with sufficient  $\text{Ca}(\text{OH})_2$  suspension, dry, char, and heat at 600° for 2–3 hours. Transfer ashed material to still, which contains several beads and sufficient  $\text{AgClO}_4$  to precipitate  $\text{Cl}_3$  with 20 ml of the distilling acid ( $\text{HClO}_4$  or  $\text{H}_2\text{SO}_4$ , depending on Ca content of sample) as directed in (a) and continue as directed in (b)(1) "Distil at 135–140°, etc."

*Certain organic phosphatic materials* (small samples of bone, 2–5 g, such as entire bones of small test animals) in which quantity of organic matter is not excessive, may be placed in still and distilled directly as directed in (b) (1) without preliminary ashing. If sample contains appreciable Ca (bone samples), use  $\text{HClO}_4$ , with reasonable precaution; if the organic phosphatic material does not contain excessive Ca, use 1+1  $\text{H}_2\text{SO}_4$ . In either case add more  $\text{Ca}(\text{OH})_2$  to first distillates and ash for longer period of time in order completely to destroy distilled organic matter (fatty acids). Transfer contents of dish to freshly prepared still (29.27) with the 20 ml of  $\text{HClO}_4$  as directed in (a) and proceed with the final distillation (29.27).

*Baking powders* (Ca phosphate and combination types) are handled as follows: Place 10 g of sample in deep, covered Pt dish or casserole and slake cautiously with ca 20 ml of the  $\text{Ca}(\text{OH})_2$  suspension. After action subsides, rinse cover, dry contents of dish *thoroly*, and ash at 600° for 2–3 hours. After completing the ashing step, cool dish and, because of excess of carbonate in the ash, treat it with several small portions of warm  $\text{H}_2\text{O}$ , breaking up with flattened stirring rod, and transfer leachings to still. Then transfer remaining contents of dish with the 20 ml of distilling  $\text{HClO}_4$ , avoiding excessive effervescence when acid is added to carbonate soln in still. Add several glass beads and sufficient  $\text{AgClO}_4$  soln, and proceed as directed in (b) (1) "Distil at 135–140°, etc." With *combination* or *Na Al sulfate* baking powders, collect at least 400 ml of preliminary distillate, (b) (4).

By use of specially trapped still it is possible to analyze highly phosphatic *inorganic* or *thoroly* ashed materials, and phosphoric acids, with a single distillation. The special trap, or scrubber, consists of 12–15 g of small, hollow glass beads supported in side-neck of the 125 ml Claisen flask by several indentations punched in side wall, and capped by glass disk or inverted bottom of a 15 mm test tube. After construction of the glass-bead scrubber the side-neck is sealed off immediately above outlet tube. (Beads in the scrubber must be wet with a little  $\text{H}_3\text{PO}_4$  before distillation to furnish a liquid acid phase.) Take 20 ml of sirupy  $\text{H}_3\text{PO}_4$ , by itself, and 10 g samples of Ca phosphate with 20 ml of the  $\text{HClO}_4$ , for the distillation, and collect at least 400 ml of distillate at 135°. With the single distillation observe the precautions outlined in 29.24, and also in 29.27 regarding neutralization of final distillates. (Distillates should show practically negligible acidity.) Presence of only *traces* of distilled  $\text{H}_3\text{PO}_4$  will vitiate the titration; as little as 20 micrograms of  $\text{P}_2\text{O}_5$  will definitely interfere. Accordingly, if the single distillation procedure is to be applied with confidence, it is necessary to test the distillates obtained from phosphatic materials, by

means of the special still, for the presence of this interference. For a convenient test utilizing Schricker's reagent (12), add 5 ml of a 1+9 dilution of this reagent to 45 ml of distillate in 50 ml cylinder or Nessler tube, mix, and immerse in steam bath for 5-10 min. Compare against a blank by sighting down tube. Blue or blue-green color denotes phosphate, and as little as 5 micrograms (as  $P_2O_5$ ) is readily detected. If distillate shows traces, make sure that such quantities are below an interference level of 15 micrograms in the titration aliquot before titrating additional portions of distillate. (The test with Schricker's reagent is also useful in the usual double distillation procedure where a phosphate interference is possible. Use of the special trap will save time where highly phosphatic materials are handled as routine, but it is not justified in ordinary work because of poor efficiency owing to excessive refluxing in the distillation.)

(3) *Excessively fatty and oily food materials* (oil-packed foods, certain meats, etc., also entire undried and unground organs of test animals), where there is danger of F loss thru incomplete wetting with the  $Ca(OH)_2$  fixative soln, may be handled as follows: Weigh appropriate quantity of sample, usually 10-25 g, into still, and add Ag (preferably 0.1-0.2 g of solid  $Ag_2SO_4$ ), several glass beads, and 20-25 ml of the 1+1  $H_2SO_4$ . Distil at 130-135° and collect 200-250 ml distillate in a beaker or open vessel. If foaming is excessive, increase quantity of distilling acid, and where necessary use a larger (250-300 ml) still. If a larger still or more acid is used, collect proportionately more first distillate. (Oil or fat of many of these products will tend to prevent foaming; and in some instances, use of a piece of purified paraffin about the size of a pea is additional aid.) Oxidize distillate in the cold by cautious addition of 2-3 ml of *F-free* 30%  $H_2O_2$  to remove sulfites, allow to stand a few minutes, and evaporate portionwise in Pt dish containing an excess (10-15 ml) of the  $Ca(OH)_2$  suspension. Ash residue at 600° until clean. Proceed as directed in (b) (1), beginning "Transfer contents of dish to freshly prepared still, etc." Handle pure oils by similar procedure; with these products use 10 g sample with 25 ml of 1+1  $H_2SO_4$  and carry temp. at first to ca 170° to saponify; then carefully bring temp. down to 140° with distilling  $H_2O$  and collect 250 ml or more of distillate. (It will probably be necessary to use a higher reading thermometer for this procedure.) Oxidize distillate with 30%  $H_2O_2$  and evaporate to dryness after adding excess of the  $Ca(OH)_2$  suspension. Ash at 600° and after brief preliminary ash period remove dish, add a little  $H_2O$  plus an additional 1-2 ml of the  $H_2O_2$  to remove sulfides, dry, and complete ashing. Proceed as directed previously in this paragraph.

(4) *Aluminum and boron compounds* repress the evolution of F, and complete isolation of their F content necessitates a preliminary distillation at elevated temp. For this purpose, weigh sample, usually 5-10 g, into still, add 25 ml of the 1+1  $H_2SO_4$ , and conduct first distillation at 160-165° (special thermometer), collecting 300 ml of distillate. Oxidize distillate with 30%  $H_2O_2$  as above, evaporate down in Pt with excess of  $Ca(OH)_2$  suspension, incinerate briefly at 600°, and proceed as directed in (b) (1), beginning, "Transfer contents of dish to freshly prepared still, etc."

## 29.27

### FINAL DISTILLATION

The final distillation is always made from  $HClO_4$ , and due precautions are taken to secure a low acid distillate, 29.24(a). Interferences, such as organic matter, phosphate, sulfate, etc., must be absent in distillate, hence it must be made with careful temp. control in presence of enough Ag salt to repress  $HCl$  evolution (29.23). It is well to check distillates for presence of possible phosphate as directed in 29.26(b) (2), and where advisable, as in (b) (4), to test for sulfate with a little dilute  $BaCl_2$  soln.

The  $\text{HClO}_4$  used in final distillation is usually employed in transferring ash to still, 29.26(a). A few acid-alkali washed beads are used to control bumping. (Use of powdered  $\text{SiO}_2$  does not appear necessary for the microdetermination.)

In order to promote better recoveries, and to minimize and render constant the distillation blank discussed in 29.23 and 29.28, prepare the still by special cleaning process before this transfer by treating it with *hot* 10%  $\text{NaOH}$  soln after each run, flushing out with tap  $\text{H}_2\text{O}$ , and then rinsing with distilled  $\text{H}_2\text{O}$ . Occasionally (at least once a day, and especially after it has stood idle for any length of time), treat the still additionally by boiling down 15–20 ml of the 1+1  $\text{H}_2\text{SO}_4$  until it is filled with fumes. Pour off the acid, treat with the 10%  $\text{NaOH}$  soln, and *thoroly* rinse out. (The cleaning should be especially meticulous after high F or high  $\text{SiO}_2$  samples have been distilled, and in such cases the condenser should also be cleaned.)

At this stage the prepared sample has been transferred to the specially treated still, as directed above, for the final isolation of F. Begin distillation, and when temp. reaches  $137^\circ$  maintain at this point ( $\pm 2^\circ$ ) by adding  $\text{H}_2\text{O}$  from the dropping funnel, 29.24(a). Heat the still at such a rate that all distillations require about the same amount of time. This promotes uniformity in the blank correction. Catch distillate in 150 ml volumetric flask, or, if one of these is not at hand, use a 200 ml flask. After a few ml of distillate have been collected, add 1–2 drops of the *p*-nitrophenol indicator, and keep distillate alkaline to this indicator (faintest perceptible yellow) by adding a drop or two of the 0.05 *N*  $\text{KOH}$  from 10 ml buret from time to time during distillation, swirling receiver contents. Regulate this addition of alkali so that distillate is neutralized (within 1 drop of alkali) as it approaches the mark. Note carefully volume of alkali used. Make distillate to mark and mix *thoroly*. Do not allow a F distillate to stand more than a few minutes before making neutral.

If sample contains such large quantities of Cl that bumping in still cannot be controlled, dissolve the ash of another sample, and acidify *slightly* with  $\text{HClO}_4$ . Dilute considerably and precipitate Cl in dish with the  $\text{AgClO}_4$  soln, avoiding large excess. Filter thru glass filter, wash precipitate *thoroly* with hot  $\text{H}_2\text{O}$ , and evaporate filtrate and washings to dryness after adding excess (to alkalinity) of the  $\text{Ca}(\text{OH})_2$  suspension. Transfer residue to still with the  $\text{HClO}_4$  and repeat distillation as directed above.

## 29.28

### TITRATION

Place aliquot of final distillate in Nessler tube and mark "S" (sample). (Optimum quantities of F to be titrated are 60–70 micrograms for the 100 ml Nessler tubes and 30–40 micrograms for the 50 ml size, and it is well to make exploratory titration on small aliquot to check approximate F content of distillate. The larger tubes are necessary if precise results on low-F foods are to be obtained.) Add the 0.05 *N*  $\text{HCl}$ , 4.00 ml for the 100 ml tubes and 2.00 ml for the 50 ml size, and 1.00 ml of the hydroxylamine soln. (For routine work *with the 100 ml tubes* the acid and hydroxylamine can be blended as 0.04 *N*  $\text{HCl}$  made to 0.2% W/V strength with hydroxylamine hydrochloride, and the proper quantity of both reagents added to the tubes in a single operation with a 5 ml pipet.) Dilute to ca 90 (or 40) ml, mix well, then add the proper amount of the alizarin indicator (2.00 or 1.00 ml) and mix again. Always add and mix in the hydroxylamine before adding the indicator. Prepare a blank tube ("B") by adding proper amount of  $\text{HCl}$  and hydroxylamine, and a quantity of the 0.05 *N*  $\text{KCl}$  soln representing same proportion of total volume of 0.05 *N*  $\text{KOH}$  used to neutralize distillate as the aliquot volume taken for the sample tube represents of the total distillate volume. (Thus, if 1.00 ml of 0.05 *N*  $\text{KOH}$  was used to neutralize a distillate volume of 150 ml and the aliquot taken for tube "S" was 75 ml, add 0.5



ml of the 0.05 *N* KCl to tube "B.") Dilute and mix, allowing slightly more head-space than in sample tube. Then add proper volume of alizarin indicator and mix.

Measure the Th soln into tube "S," mixing between additions, until end point of about proper shade is reached. Dilute to mark, mix, and check this end-point shade. Note from curve, 29.25(1), approximate volume of standard F soln corresponding to this volume of Th soln, and add ca 0.5 ml *less* than this quantity of standard F soln to "B." Mix in, then add exactly the same volume of Th soln as was added to "S," duplicating approximately the increments in which it was added and the number of mixings. Dilute nearly to mark and compare colors of "S" and "B." (If volume of standard F soln added to "B" was properly chosen, this tube should be only slightly pinker in shade than sample tube.) Bleach the "B" tube to exact match with tube "S" by adding more of the standard F soln to the former in increments of 1-2 drops, mixing gently between additions. Make to mark for final comparison and observe usual precautions of allowing bubbles to subside and of transposing tubes when final comparisons are made. (At the match-point, F content of tube "S" equals quantity added to tube "B.") Check this end point by adding 1-2 drops in excess of standard F soln to tube "B." A distinct over-bleach should develop.

Repeat the titration on aliquots of different size to obtain total quantity of F distilled. If time is available, repeat entire determination with different weight of sample.

*For precise work*, evaluation of the reagent and of the distillation blank is necessary, 29.23. Determine distillation blank by making several distillations with the prescribed quantities of  $\text{HClO}_4$  and  $\text{AgClO}_4$  solns from the freshly cleaned still, titrating the distillate as directed above with as large an aliquot as practicable. Average of values found should not be greater than 2-3 micrograms of F. If quantities found by individual blank runs are too small to be determined accurately, make five or more separate distillations and evaporate distillates, 150 ml each time, successively in same Pt dish for a final distillation and average blank figure. The distillation and total determination blanks can usually be *combined* by carrying a run (with same quantities of reagents and similar evaporation and ashing treatment) thru entire procedure. Reagents and manipulations should increase the distillation blank but little.

Calculate total quantity of F distilled from quantity found in aliquot titrated, subtract proper blank, and refer net figure to weight of sample taken. If double distillation procedure was used, make appropriate correction.

## RAPID METHOD RESTRICTED TO FLUORIDE RESIDUES ON APPLES AND PEARS

### 29.29

#### PRINCIPLES

This method utilizes the acid filtrate from the strip soln of apples and pears when prepared *with HCl rinse and acidification*, 29.49. An aliquot of filtrate is oxidized colorless with  $\text{KMnO}_4$ , the soln is then reduced with hydroxylamine, and a back titration is conducted upon a sub-aliquot in Nessler tubes;  $\text{Zr}(\text{NO}_3)_4$  is used in the titration, with purpurin (1, 2, 4, trihydroxyanthraquinone) as indicator. The principle of the back-titration, as applied here, is similar to that employed in the general method where  $\text{Th}(\text{NO}_3)_4$  and alizarin occupy similar roles. The method is based, in general, on the work of Kolthoff and Stansby (13). Provision is made for the removal of interfering anions, and the high acidity employed in the titration minimizes interference of metals that would otherwise lake with the indicator.

## 29.30

## APPARATUS

- (a) *Volumetric flasks*.—50 ml.  
(b) *Nessler tubes*.—50 ml glass-stoppered, tall form, matched for height and color (see 29.33).

## 29.31

## REAGENTS

- (a) *Mixed nitrate soln*.—Dissolve 3.0 g of  $\text{Ba}(\text{NO}_3)_2$  and 2.0 g of  $\text{Th}(\text{NO}_3)_4 \cdot 4\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and make to 100 ml.  
(b) *Potassium permanganate soln*.—Saturated; ca 6% W/V.  
(c) *Hydroxylamine hydrochloride soln*.—5% W/V.  
(d) *Ferrous chloride soln*.—Dissolve ca 1.0 g of Fe powder or wire in 50 ml of 1+1 HCl, dilute, and filter into 500 ml volumetric flask. Add a few ml of the hydroxylamine hydrochloride soln and make to mark. Dilute still further, before use, if desired.  
(e) *Purpurin indicator*.—0.01% W/V in alcohol. Dissolve 25 mg of the pure dye in alcohol, heating if necessary, and make to 250 ml with the same solvent. *Prepare fresh weekly*.  
(f) *Zirconium nitrate soln*.—Dissolve 1.50 g of  $\text{Zr}(\text{NO}_3)_4 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ , acidify with 20 ml of HCl, and dilute to 1 liter. Filter if not clear.  
(g) *Standard fluoride soln*.—So dilute a stock soln of pure NaF that 1 ml = 54.5 micrograms of F.

## 29.32

## DETERMINATION

Place 20 ml of the well-mixed acid strip filtrate, 29.49, in 50 ml volumetric flask. Add 2.0 ml of the mixed nitrate soln, then 4.0 ml of the  $\text{KMnO}_4$  soln. Rinse down neck of flask with a little  $\text{H}_2\text{O}$  and place on active steam bath for 5 min. Remove flask, and while still hot, add the  $\text{NH}_2\text{OH} \cdot \text{HCl}$  soln from buret, slowly and with swirling, until  $\text{MnO}_2$  is discharged and soln is colorless. Add ca 0.5 ml of this reagent in excess. (Appreciable phosphate is revealed as flocculent  $\text{Th}_3(\text{PO}_4)_4$ , and sulfate as the precipitate with Ba. Sometimes  $\text{KMnO}_4$  is occluded in this sulfate and/or phosphate precipitate and a pink color tends to persist but does not interfere.) Cool, make to mark, and filter. (Filtrate must be clear. If there is perceptible turbidity, return filtrate thru filter several times if necessary, until filtrate is *brilliant*.) Pipet 25 ml of clear filtrate into Nessler tube and mark "S."

For blank or comparison tube use 25 ml of "blank" soln containing the reagents as used in the method and prepared as follows:

Make 50 ml of 10% sodium oleate soln, 29.36(e), 50 ml of 30% W/V NaOH soln, and 15 ml of HCl to 1 liter. Acidify portions with one-tenth volume of HCl as if the soln were an actual "strip," and filter, returning filtrate thru filter until perfectly clear. (Chilling the soln and shaking vigorously will "churn" the precipitated oleic acid and aid in obtaining clear filtrate.) Carry 20 ml portions of acidified filtrate thru procedure exactly as directed above. (It is best, in order to duplicate more closely conditions of actual determination, to use the 50 ml volumetric flasks and 20 ml aliquots in preference to working up larger aliquots with correspondingly larger amounts of reagents. After being made to volume and filtered, the blank solns may be combined to form a supply of "blank." Ten portions worked up as above yield ca 500 ml of blank, or enough for almost 20 determinations.)

Add 25 ml of this "blank" to second Nessler tube ("B"), and to both tubes "S" and "B" add 15.0 ml of HCl measured as carefully as possible from graduate. (Al-

ways add acid to soln instead of vice versa.) Mix, and match tubes for color. The "S" tube will usually be found to have slight greenish tint in comparison with the "B" tube, due presumably to traces of Fe. Balance both tubes to same shade by adding the  $\text{FeCl}_2$  soln dropwise to the appropriate tube and mixing. *This operation must be carefully done.* When there is no discernible difference in tint, add exactly 1.00 ml of the purpurin indicator to each tube. Mix, then add to each, 1.50 ml of the Zr soln from 10 ml buret and mix. Do not shake tubes violently when mixing in reagents; 4 or 5 gentle inversions are sufficient. Observe color difference, if any, between the two tubes when looking down their length towards a white reflecting surface. If there is no appreciable difference *after 5 min.*, F content of sample is negligible. If color of tube "S" is yellower, presence of F is indicated. In this case, add additional amounts of the  $\text{Zr}(\text{NO}_3)_4$  soln to tube "S" until its color matches approximately that of tube "B" (to nearest 0.5 ml of the Zr soln). Dilute "S" to mark and mix. Now add to "B" exactly same total volume of Zr soln as was added to tube "S," mix, and allow tube to stand for 2 min. for lake to develop fully. Back-titrate the standard fluoride soln into "B" from 10 ml buret until tubes match, frequently mixing, and making nearly to volume as end point is approached. Add the F in increments of ca 0.1 ml at this stage, and observe usual precautions of transposing tubes and allowing bubbles to subside when making comparisons. Make to mark for final comparison. Check the end point by adding 0.1–0.2 ml of the standard F soln in excess. A distinct overbleach should develop. For sample weight of one kilogram and aliquots prescribed above, each ml of standard F soln consumed in back-titration is equivalent to a F content on the fruit sample, *removable by the solvent treatment*, of 3.0 p.p.m. Correct result obtained in titration by the sample weight ratio. (Thus, a titer of 3.27 ml of the standard F soln, with a 1.40 kilogram sample (ca 10 fruit) represents a F content of 7.0 p.p.m. The volume restrictions of a 50 ml Nessler tube will allow the estimation of a spray residue content up to ca 11 p.p.m. F.) If calibration mark is exceeded in the back-titration, use a 10 ml aliquot of the acid filtrate in tube "S," and make to 25 ml with "blank" soln, correcting titer of standard F soln by appropriate factor.

## 29.33

## NOTES ON RAPID METHOD

Glass-stoppered Nessler tubes are almost essential with the strong acid prescribed in this determination and are likewise much to be desired in the general method for F with Th and alizarin, 29.28. Analysts familiar with the Th-alizarin back-titration procedure should have no difficulty with the Zr-purpurin titration. With the latter, however, color changes are not so apparent and titration is less sensitive. However, with careful work, results accurate to at least 0.5 p.p.m. may be expected. Indicator color at prescribed acidity is yellow, and fully laked indicator is orange red. This contrasts with the Th titration where the corresponding range is from a yellowish green to reddish purple. Hence in the present method choice of an end point involves discrimination between varying shades of orange. The addition of the 1.50 ml of Zr soln to tube "B" at the start is merely to provide an intermediate shade of orange to guide the analyst in amount of Zr to be added to tube "S." Analysts may prefer to work with redder or yellower end point shade. In any event, it is urged that a number of titrations be made by adding various quantities of the standard F soln as unknowns to Nessler tubes and carrying thru a back-titration as directed above, for purpose of learning the color changes involved. Pure aqueous solns instead of "blank" may be used with acidities of 20 ml of  $\text{HCl}/50$  ml.

Accuracy of results with the rapid method presupposes complete removal of

spray residue F by the solvent process and good accuracy (not necessarily precision) in the titration. These conditions may not always obtain; unless carefully done, the solvent method may not be entirely effective, and results on strip solns containing known quantities of F have tended slightly low. Hence accuracy above 95% is not to be expected with this procedure.

#### LEAD (14)—TENTATIVE

##### 29.34

##### PRINCIPLES

The general method calls for ashing, 29.37, separation of the Pb, either as the dithizone complex, 29.39, or as the sulfide, 29.40, followed (depending upon quantity) by electrolytic determination, 29.41, or by colorimetric dithizone determination, 29.42, in comparator tubes, or with a photometer. The subject of interference is treated separately, 29.43–29.45, and the analyst should familiarize himself with the details of these sections before applying the method. Special methods of sample preparation are presented under 29.46–29.49.

##### 29.35

##### PRECAUTIONS

The analyst should decide whether nature of determination requires unusual care in purification of reagents, or whether blank determination will be sufficient. The smaller the quantity of Pb to be determined, the greater the care required in reduction of blank (*see also* 29.42).

To test suitability of reagents place 15–20 ml of concentrated acids or 10–15 g of solid reagents dissolved in redistilled  $H_2O$  in separator and add sufficient Pb-free citric acid to prevent precipitation by  $NH_4OH$  of Fe, Al, alkaline earth phosphates, or other substances. Make soln ammoniacal and add 2–3 ml of 10% KCN soln. Shake soln with ca 5 ml of dithizone soln, 29.36(e) (5–10 mg/liter). If lower layer is green, transfer it to another separator and extract excess dithizone with  $NH_4OH$  (1+99) to which has been added a drop of KCN soln. If  $CHCl_3$  layer is colorless, consider test negative for general analytical purposes.

When special purification becomes necessary, redistil  $H_2O$  (distilled  $H_2O$  stored in Sn-lined tanks usually contains Pb and Sn),  $HNO_3$ , HCl, HBr, Br, and  $CHCl_3$  (U.S.P. free from chlorides) in all-glass stills (Pyrex is preferred.  $NH_4OH$  is prepared by distilling the ordinary reagent into ice-cold redistilled  $H_2O$ ). If stills are new, steam them out with hot HCl or  $HNO_3$  vapors to remove "surface" Pb. (Subsequent distillates may not be totally Pb-free.) Purify citric acid, Na or  $NH_4$  acetate,  $Al(NO_3)_3$ ,  $Ca(NO_3)_2$ , and  $Na_2SO_4$  by precipitating the Pb from their aqueous solns with  $H_2S$ , using 5–10 mg of  $CuSO_4$  as coprecipitant (citric acid and  $Al(NO_3)_3$  solns require adjustment with  $NH_4OH$  to pH 3.0–3.5, bromophenol blue indicator). Filter (fritted glass filter is most convenient), boil filtrates for 20 min. to expel excess  $H_2S$  and filter again if necessary to obtain brilliantly clear solns. Purify other reagents by recrystallization.

Store redistilled acids or purified solns of reagents in resistant glass containers of minimum Pb content (Pyrex is suitable), carefully cleaned of surface Pb with hot  $HNO_3$ . Paraffin-lined bottles may be used for alkaline reagents.

Clean new glass and chemical ware carefully with hot 10% NaOH soln followed by hot  $HNO_3$ , and use only for Pb determinations.

In preparation of samples for analysis, avoid Pb contamination. If mixing or grinding is necessary, use porcelain mortar if possible. Avoid use of metal food grinders unless previous experiment has shown that no contamination of sample

with Pb or Sn results. If product to be analyzed cannot be thoroly mixed in its own container, or if composite sample of a number of containers is desired, empty into large glass jar or porcelain dish and mix thoroly with wooden spoon or porcelain spatula. If liquid portion of sample cannot be incorporated into ground solid material to obtain homogeneous mixture, analyze separately. If the food is packed in tins having soldered seams (sardines and meats), open tins from bottom to avoid contaminating sample with bits of solder. Avoid sifting in preparation of samples to prevent metallic contamination or segregation of Pb.

### GENERAL METHOD

#### *Sn and Bi Absent*

(Applicable to such materials as carbohydrates, cereals and cereal products, cacao and dairy products, feeds, meats, fish, plant material, fruit and fruit products, fresh vegetables, etc., and in general to all organic materials (except fats) in which no Sn and Bi are encountered. For products containing Sn (canned foods) or Bi, proceed as directed under 29.43–29.45.)

### 29.36

#### REAGENTS

(a) *Standard lead solns.*—Dissolve 20–50 g of C.P.  $\text{Pb}(\text{NO}_3)_2$  in minimum of hot  $\text{H}_2\text{O}$  and cool with stirring. Filter crystals with suction on small Büchner funnel, redissolve, and recrystallize. Dry crystals at 100–110° to constant weight. Cool in desiccator and preserve in tightly stoppered bottle. (Product has no  $\text{H}_2\text{O}$  of crystallization and is not appreciably hygroscopic.) Prepare stock soln containing equivalent of 2 mg of Pb (3.197 mg of  $\text{Pb}(\text{NO}_3)_2$ ) per ml in 1%  $\text{HNO}_3$ , (b). Prepare weaker dilutions with 1%  $\text{HNO}_3$  as needed.

(b) *Nitric acid.*—1%. Dilute 10 ml of fresh, water-white  $\text{HNO}_3$  (sp. gr. 1.40) to 1 liter with redistilled  $\text{H}_2\text{O}$ . If acid has been redistilled, boil off nitrous fumes before making dilution.

(c) *"Ash Aid" soln.*—Dissolve 40 g of  $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  + 20 g of  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  in 100 ml of  $\text{H}_2\text{O}$ .

(d) *Citric acid.*—Concentrated Pb-free soln. 1 ml = 0.5 g of citric acid (reagent partially neutralized with  $\text{NH}_4\text{OH}$  during purification, 29.35).

(e) *Diphenylthiocarbazone (dithizone).*—Dissolve ca 1 g of the commercial reagent in 50–75 ml of  $\text{CHCl}_3$  and filter if insoluble material remains. Shake out in separator with four 100 ml portions of metal-free (redistilled)  $\text{NH}_4\text{OH}$  (1+99). Dithizone passes into aqueous phase to give orange colored soln. Filter aqueous extracts into large separator thru pledget of cotton inserted in stem of a funnel. Acidify slightly with dilute  $\text{HCl}$  and extract precipitated dithizone with two or three 20 ml portions of  $\text{CHCl}_3$ . Combine extracts in separator and wash two or three times with  $\text{H}_2\text{O}$ . Draw off into beaker and evaporate the  $\text{CHCl}_3$  with gentle heat on steam bath, avoiding spattering as soln goes to dryness. Remove last traces of moisture by heating for 1 hour at not over 50° in vacuo. Store dry reagent in dark in tightly stoppered bottle. Make up reagent solns for extraction to contain 100, 50, and 10 mg/liter in freshly redistilled  $\text{CHCl}_3$  (15) and store in dark at 5–10°. (A stock soln of dithizone in  $\text{CHCl}_3$  containing 1 mg per ml will keep a long time and is convenient for use in making dilutions.)

(f) *"Stripping" reagent.*—To 20 ml of saturated Na acetate soln, add 10 ml of acetic acid and make to 100 ml.

(g) *Potassium iodide soln.*—2%. Prepare as frequently as is necessary to prevent formation of a starch-I color when mixed with reagent (f) in proportions specified in 29.41(c).

(h) *Starch soln.*—Make up 1 g of soluble starch to 200 ml.

(i) *Sodium thiosulfate.*—Approximately 0.1 *N* stock soln. Dissolve 24.8 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in 1 liter of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  and allow to stand (preferably for 2 weeks) before use. Prepare ca 0.001 and 0.005 *N* solns by dilution of the stock soln in exact ratios of 1:100 and 1:20 with  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  and standardize these electrolytically, using standard Pb soln equivalent to 0.2–1.0 mg of Pb for the 0.001 *N* dilution and 1–5 mg of Pb for the 0.005 *N* dilution. Subtract anode blanks, 29.41 (b) and (c), and take as the  $\text{Na}_2\text{S}_2\text{O}_3$  factor the average number of mg of Pb equivalent to 1 ml of the solns. Make fresh dilutions daily and check Pb factor at least every month.

(j) *Ammonia-cyanide mixture.*—To 100 ml of 10% recrystallized, phosphate-free (16) KCN in 500 ml volumetric flask add sufficient redistilled  $\text{NH}_4\text{OH}$  to introduce 19.1 g of  $\text{NH}_3$ , and complete to volume with redistilled  $\text{H}_2\text{O}$ . (Concn. of redistilled  $\text{NH}_4\text{OH}$  can be determined by sp. gr. or titration.)

(k) *Pure metallic tin.*—Purest obtainable, such as Bureau of Standards Sample No. 42 B (0.0035% Pb). Granulate Sn as finely as possible by melting and pouring very slowly into  $\text{H}_2\text{O}$ . Determine Pb content as follows: Dissolve 1–2 g sample in HBr or HCl and volatilize the Sn by evaporating soln to dryness and treating with several 5 ml portions of the HBr- $\text{Br}_2$  mixture, (l), evaporating to dryness on steam bath after each treatment. Take up with 2–3 ml of  $\text{HNO}_3$ , evaporate to dryness to expel Br, and take up with hot  $\text{H}_2\text{O}$ . Filter, adjust acidity to 1% with  $\text{HNO}_3$ , and proceed as directed in 29.41.

(l) *Hydrobromic acid-bromine mixture.*—To 250 ml of 40% redistilled HBr add 35 ml of redistilled liquid Br.

(m) *Sodium polysulfide.*—Dissolve 480 g of  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  and 40 g of NaOH in  $\text{H}_2\text{O}$ , add 16 g of powdered S, shake until S dissolves, filter, and dilute to 1 liter.

(n) *Hydrochloric acid-citric acid soln.*—Add quantity of Reagent (d) equivalent to 50 g of citric acid to 50 ml of HCl and dilute to 250 ml.

(o) *Sodium oleate soln.*—10%. To 45 ml of 30% NaOH soln and 400 ml of  $\text{H}_2\text{O}$  in 1.5 liter beaker, add slowly, while heating and stirring, 90 g (by difference from separator) of oleic acid. Heat mixture on steam bath until soap is entirely dissolved. (Small flocculent precipitate of impurities may remain.) Cool, dilute to 1 liter, mix, and filter.

(p) *Ammonia-cyanide-citrate soln.*—Dissolve 10 g of phosphate-free KCN and 10 g of citric acid in 250 ml of  $\text{NH}_4\text{OH}$  (sp. gr. 0.90) and dilute to 1 liter. Reagent is conveniently preserved in dispensing apparatus that will minimize loss of  $\text{NH}_3$  by volatilization.

## 29.37

### PREPARATION OF SAMPLE (ASHING)

Quantity of material taken for sample depends upon amount available and expected Pb content, and whether the Pb is to be determined as directed in 29.41 or 29.42. In general, weigh representative sample of 5–200 g, depending upon conditions, into porcelain dish or casserole of convenient size. Dry wet samples on steam bath or in oven. Add 2–5 ml of the “ash-aid” soln, 29.36(c), to products difficult to ash (meats), or to furnish ash bulk to low ash products (candies, and jellies low in fruit content); mix well, and dry. Char gelatin, carbohydrate foods such as jam, and other products that have a tendency to swell excessively, by carefully heating over burner. (Swelling can be controlled by playing small flame from glass jet over surface of material in dish, but a metallic burner must not be used for this purpose because of possible metallic contamination.) Do not allow material to ignite. Milk, candies, etc., may be charred without ignition by adding sample a little at a time to casserole heated over burner or hot plate. (An overhead radiant heater is often very

convenient.) When samples are dry or charred, place in temp.-controlled muffle and raise temp. *slowly* to 500° without ignition. If sample contains fat, "smoke" it away by heating sufficient length of time at ca 350°. Cover floor of muffle with piece of asbestos board or SiO<sub>2</sub> plate so that sample receives most of its heat by radiation from sides and roof and not by conduction from hotter floor of muffle.

If muffle is provided with automatic control, conduct ashing overnight at not over 500°. If sample is not completely ashed the next morning or if day-time ashings at 500° are not proceeding satisfactorily, remove casserole, cool, and moisten char with 2-5 ml of the ash-aid. Dry contents of casserole past danger of spattering (no free liquid) and replace it in muffle. If ashing is not complete or proceeding rapidly after 30 min., remove casserole, cool, and cautiously add 2-3 ml of HNO<sub>3</sub>. Dry, place in muffle, and continue ashing until practically C-free. Avoid excessive use of ash-aid and particularly HNO<sub>3</sub>, if sample still contains much intermixed C, because local overheating or deflagration may result, especially if much K is present in ash.

When a clean ash is obtained, cool, cover casserole with watch-glass, and add cautiously 15-20 ml of HCl. Rinse down watch-glass with H<sub>2</sub>O and heat on steam bath. If a *clear* soln is not obtained, evaporate again to dryness and repeat addition of HCl. If insoluble matter persists, evaporate HCl and dehydrate SiO<sub>2</sub> by heating to fumes with 5-10 ml of 60% HClO<sub>4</sub> (double distilled preferred). If HClO<sub>4</sub> is used, considerable H<sub>2</sub>O (200 ml) may be necessary to completely dissolve KClO<sub>4</sub> later as when KCN is used in the dithizone extraction of Pb, 29.39.

Dilute with H<sub>2</sub>O and filter soln when necessary with suction thru fritted glass filter of fine porosity. Catch filtrate in 500 ml glass-stoppered Erlenmeyer flask under bell-jar. Leach insoluble material on filter successively with a few ml of hot HCl, the hot HCl-citric acid soln, and hot 40% NH<sub>4</sub> acetate soln.

In certain instances take following special precautions:

(1) If quantity of insoluble material (SiO<sub>2</sub>) remaining on filter is abnormal, flush it into Pt dish with H<sub>2</sub>O, evaporate, and treat residue with one or two 5 ml portions of HF. Evaporate to dryness and take up residue with H<sub>2</sub>O and a few drops of HCl or HClO<sub>4</sub>, and add to bulk of ash filtrate. (2) When ashing is of long duration, no ash-aid has been used, or natural ash is low with little ash bulk, Pb may be baked on dish. To remove this Pb, add a few pellets (2-3 g) of NaOH and dissolve in a few ml of hot H<sub>2</sub>O. Tilt dish so that sirupy soln completely wets that portion of interior originally occupied by sample, then heat for short time on steam bath, but do not bring to dryness. (Overheating with strong NaOH may result in extracting a few micrograms of Pb from casserole. Porcelain retains Pb to a less extent than does SiO<sub>2</sub> but may contain very small quantities of Pb.) Take up residue with H<sub>2</sub>O and add directly to filtrate. Finally rinse dish with a few ml of hot HCl followed by hot H<sub>2</sub>O.

## 29.38

### ISOLATION OF LEAD

Procedure 29.39, while rapid and convenient, is limited to those materials which, with the aid of citric acid, will yield the clear ammoniacal soln demanded for quantitative extraction of Pb with dithizone. Pb is readily occluded by many alkaline precipitates (Mg and Ca phosphates, Al and ferric hydroxides and silicates). Many food materials may be handled in this way as the naturally occurring amounts of these substances are not excessive. However, some materials contain more of these substances than can be kept in soln under alkaline conditions with any reasonable amount of citric acid (17). In these cases proceed as directed under 29.40. Difficulty of ammoniacal precipitation may sometimes be overcome by limiting sample size in those cases where sampling is no problem.

## 29.39

*Dithizone Extraction*

(Applicable to most carbohydrates and cereal foods, fruit and fruit products, milk, fresh vegetables, plant materials, etc.)

Transfer the ash soln to 300 ml short-stemmed separator and add citric acid reagent, 29.36(d), equivalent to 10 g of citric acid. Make slightly alkaline to litmus with  $\text{NH}_4\text{OH}$ , keeping soln cool, and allow to stand 1–2 min. If precipitate forms, redissolve with  $\text{HCl}$  and isolate the Pb as directed under 29.40. If no precipitate forms, add 5 ml of 10%  $\text{KCN}$  soln (more may be necessary if large quantities of Zn, Cu, Cd, etc., are present) and check pH of soln by adding a drop of thymol blue and observing color of drop. (The pH should be 8.5 or above, blue-green to blue with thymol blue.) If ash was highly colored with Fe, keep pH of soln comparatively low, because pH of 10 or above in presence of Fe may cause oxidation of dithizone. Immediately extract with 20 ml portions of dithizone reagent, using the weaker solns unless exceptionally large quantities of Pb are present. Shake 20–30 seconds, allow layers to separate, and note color of the  $\text{CHCl}_3$  phase. (The Pb dithizone complex is red, but color may be masked by excess green dithizone, giving intermediate hues of purple and crimson. Color of  $\text{CHCl}_3$  extract gives first indication of amount of Pb present, and progress of extraction can be followed by noting color of successive extracts.)

(a) *If Pb is to be determined electrolytically* ( $\text{Pb} > 0.05 \text{ mg}$ ), draw off  $\text{CHCl}_3$  layer into 125 ml short-stemmed separator containing 25–30 ml of  $\text{H}_2\text{O}$  made ammoniacal with one drop of  $\text{NH}_4\text{OH}$  (sp. gr. 0.90). Continue extraction until two successive extracts with small portions of the weaker dithizone solns show the negative green (not bluish or purple) color, combining extracts in smaller separator. Shake, allow layers to separate, draw  $\text{CHCl}_3$  fraction into another small separator, and repeat washing process as before. Draw off  $\text{CHCl}_3$  fraction as cleanly as possible into 100 or 150 ml beaker, and pass small portion of dilute dithizone soln thru separators in succession so as to wash out small portions of extract persisting in aqueous fraction. Add to beaker and evaporate  $\text{CHCl}_3$  with gentle heat on steam bath. Take up dry residue with 3–4 ml of  $\text{HNO}_3$ , and heat by swirling over low flame. Dilute to ca 25 ml and continue heating 1–2 min. in order to fume off oxides of N. Add small piece of litmus paper, neutralize with  $\text{NH}_4\text{OH}$ , dilute nearly to capacity of beaker, and add 1 ml of water-white  $\text{HNO}_3$ /100 ml of soln. Proceed as directed under 29.41(b) and (c).

*Alternative procedure.*—Draw off washed  $\text{CHCl}_3$  into separator containing 110 ml of 1%  $\text{HNO}_3$ , 29.36(b). Shake vigorously for 1 min. to decompose dithizonate and draw off green  $\text{CHCl}_3$  soln. Filter acid soln thru dry filter and pipet 100 ml aliquot into 150 ml beaker. Proceed immediately as directed under 29.41(b) and (c), taking care to heat and stir the soln to volatilize dissolved  $\text{CHCl}_3$  before adding  $\text{K}_2\text{Cr}_2\text{O}_7$  and closing electrolytic circuit. Multiply results by factor 1.1 (18).

(b) *If Pb is to be determined by colorimetric dithizone procedure* ( $\text{Pb} < 0.2 \text{ mg}$ ), do not wash the dithizone extracts with the dilute  $\text{NH}_4\text{OH}$ , but run directly into smaller separator containing 25 ml of the 1%  $\text{HNO}_3$ , 29.36(b). When extraction is complete, shake combined extracts in smaller separator and draw off green dithizone layer into another separator containing a further 25 ml portion of 1%  $\text{HNO}_3$ . Shake, allow layers to separate, and discard  $\text{CHCl}_3$  fraction. Filter acid extracts containing Pb in succession thru small pledget of wet cotton inserted in stem of small funnel, into 50 ml flask or glass-stoppered cylinder, using second acid extract to wash out separator in which the first acid extraction was made. (This procedure removes  $\text{CHCl}_3$  globules.) Make up any slight deficiency in volume with the 1%  $\text{HNO}_3$  and mix. Proceed as directed under 29.42.



## 29.40

*Sulfide Separation*

(Applicable to all products and usually necessary in the case of cacao products, tea, sardines, and all food products containing high proportion of alkaline earth phosphates, especially those of Mg, which promote formation of precipitates in ammoniacal citrate solns.)

Cool acid soln of ash, add citric acid reagent, 29.36(d), equivalent to 10 g of citric acid, and adjust to pH of 3.0–3.4 (bromophenol blue) with  $\text{NH}_4\text{OH}$ . If enough Fe is present to color soln strongly, make final adjustment with help of spot plate. (Phosphates precipitated by local action of  $\text{NH}_4\text{OH}$  may usually be redissolved by shaking and cooling.) If amount of Pb is small, add 5–10 mg of pure  $\text{CuSO}_4$  to soln to act as coprecipitant. Precipitate sulfides by passing in  $\text{H}_2\text{S}$  until soln is saturated (3–5 min.). Immediately filter with suction into flask in a bell jar (fritted glass filter of fine porosity is preferred).

(a) If Pb is to be determined electrolytically (Pb > 0.05 mg), wash flask and precipitate with a few small portions of 3%  $\text{Na}_2\text{SO}_4$  adjusted to pH 3.0–3.4 and saturated with  $\text{H}_2\text{S}$ . If a clean sulfide precipitate has been obtained, dissolve sulfides with 5 ml of hot  $\text{HNO}_3$ , wetting all portions of filter; allow to stand a few minutes and draw thru into flask in which sulfide precipitation was made. Wash filter with several portions of hot  $\text{H}_2\text{O}$ , stopper flask, shake, and boil for a few min. to remove traces of  $\text{H}_2\text{S}$ . Cool, adjust acidity to 1% with  $\text{HNO}_3$  in 100–125 ml volume and proceed as directed in 29.41(b) and (c). If there is possibility of the sulfide precipitate being contaminated with more than 3 mg of Cl, 20 mg of  $\text{As}_2\text{O}_3$ , 30 mg of  $\text{P}_2\text{O}_5$ , 50 mg of Hg, or with  $\text{Sb}_2\text{S}_3$ , dissolve as directed above with  $\text{HNO}_3$  (without previous washing with  $\text{Na}_2\text{SO}_4$  soln), wash filter with hot  $\text{H}_2\text{O}$ , and boil soln as before. Transfer to 200 ml separator, add the citric acid reagent equivalent to 5 g of citric acid, make ammoniacal, extract with dithizone soln, and determine Pb as directed under 29.39, 29.39(a), and 29.41(b) and (c).

(b) If Pb is to be determined by the colorimetric dithizone procedure (Pb < 0.2 mg), dissolve sulfides, without previous washing, with 5 ml of hot  $\text{HNO}_3$ , drawing soln thru into original flask; wash with hot  $\text{H}_2\text{O}$ , stopper, shake, and boil to remove  $\text{H}_2\text{S}$ . Transfer to 200 ml separator, add citric acid equivalent to 5 g of citric acid, make ammoniacal, extract, and determine Pb as directed under 29.39, 29.39(b) and 29.42(a) or (b).

## DETERMINATION OF LEAD

## 29.41

*Electrolytic*

(Pb 0.05–10.00 mg)

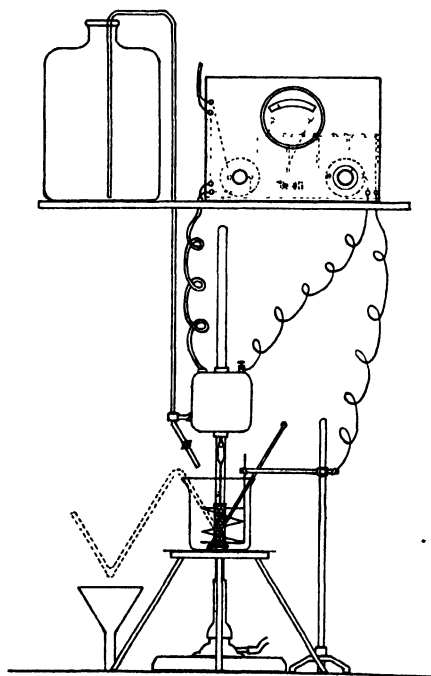


FIG. 46.—APPARATUS FOR DETERMINATION OF LEAD BY ELECTROLYTIC METHOD

(a) *Apparatus*.—See Fig. 46. Four dry cells in series constitute a convenient

source of current. The meter (0–500 milliamperes), switch, fuse, rheostat (60 ohm radio type), and variable resistance for control of the motor speed may be conveniently mounted upon a panel. Motor for rotating anode (1/20 H.P., 110 v. universal) is equipped with chuck and binding post. Rate of rotation should be sufficient to produce efficient circulation and may vary from 400 to 800 r.p.m. Electrodes consist of 45-mesh, sand-blasted, Pt gauze cylindrical anode,  $1 \times 5/16"$  and  $4"$  over-all length, and cathode of 18-gage Pt wire wound in spiral form. For larger amounts of Pb (over 5 mg) a cylindrical anode  $2 \times 1/4"$  is convenient.

(b) *Electrolysis*.—Immediately before electrolyzing bring anode to red heat in oxidizing flame of burner. (A variable titration blank is obtained if anode is not heated just before determination, due possibly to film of O adsorbed on anode and activated during electrolysis. Heating reduces and renders constant this "O blank." With small anode it will be 0.07–0.1 ml of 0.001 N  $\text{Na}_2\text{S}_2\text{O}_3$  and with larger electrode proportionately larger. Blank for a particular anode should be determined from average of series of determinations conducted on pure reagents.)

In all determinations the sample at this point is contained in volume of 100–125 ml of 1%  $\text{HNO}_3$  (with the large anode a volume of 200 ml is convenient). Place beaker (100–150 ml for small and 250 ml for large anode) in position, making sure electrodes are well covered with soln, and start motor. Heat to 60–70°, then add ca 100 mg of  $\text{K}_2\text{Cr}_2\text{O}_7$  to keep soln in oxidized state and repress formation of nitrites, especially when organic matter is present. Start current and electrolyze with ca 75 milliamperes for 20 min. at 70–80°. Use 100–150 milliamperes for larger anode. Remove flame, insert siphon in beaker, and start stream of distilled  $\text{H}_2\text{O}$  playing directly on anode. Start siphon, taking care to keep level of liquid above deposit. (A convenient siphon can also be made by connecting an inverted V-shaped tube to an ordinary water-pump.) Acid is entirely removed when current falls to zero. Turn off motor, electrolytic current, and rinse  $\text{H}_2\text{O}$ ; remove anode from chuck and give it a final rinse with  $\text{H}_2\text{O}$ .

(c) *Titration*.—Dissolve deposit in 4–5 ml of the "stripping" reagent, 29.36(f), +1 ml of the KI reagent, 29.36(g), contained in flat-bottomed vial of such size that soln just covers anode. Add a few drops of the starch soln, 29.36(h), and titrate liberated I with 0.001 N  $\text{Na}_2\text{S}_2\text{O}_3$ , 29.36(i), in the vial, using anode as stirrer and sighting down thru vial, as thru miniature Nessler tube, to detect the delicate end point. (If quantity of Pb is seen to be large (1–5 mg), use 0.005 N  $\text{Na}_2\text{S}_2\text{O}_3$  and double the amount of reagents 29.36(f) and (g). With the 2" anode still larger amounts may be used.) No yellow insoluble  $\text{PbI}_2$  should form as the deposit is "stripped"; if it does add more of the Na acetate. The deposit should dissolve completely and almost immediately. To determine amount of Pb, subtract anode and reagent blanks from the total titer and multiply by factor of the  $\text{Na}_2\text{S}_2\text{O}_3$  soln, 29.36(i).  $\text{PbO}_2 + 4\text{HI} = \text{I}_2 + \text{PbI}_2 + 2\text{H}_2\text{O}$ . Absence of interfering Bi may be assured by applying test 29.45(c).

## 29.42

### Colorimetric Dithizone (19)

(Pb 0.001–0.200 mg)

Limiting factor in determination of minute quantities of Pb by colorimetric dithizone procedure is probably size of reagent blank. The importance of careful blank determinations must be especially stressed when quantities of Pb of the order of 1–5 micrograms are being determined. With special care in purification of reagents and by use of carefully cleaned Pyrex ware, including separators, it should be possible to reduce reagent blank to 1 microgram or below. Owing to Pb-bearing dust, vapors, etc., it is necessary to expose the blank determination in muffle or on steam

bath for same length of time as sample is exposed, and to use exactly same amounts of reagents (even  $\text{H}_2\text{O}$ ) for the blank and actual determinations.

Pb is extracted from aqueous soln, under standard conditions of volume and pH, with a definite volume of a  $\text{CHCl}_3$  soln of dithizone of standard strength. Optimum pH of operation is 9.5–10.0. Dithizone strengths are so chosen that an excess of dithizone is always present in the reaction mixture. Pb is brought into the  $\text{CHCl}_3$  phase in the form of the red complex, and the uncombined green dithizone partitions between aqueous and  $\text{CHCl}_3$  phases and modifies color of extract according to relative amounts of Pb and dithizone. Thus, according to this proportion, a series of colors from red to green may be arranged with intermediate crimsons, purples, and blues. Volumes and strengths of the  $\text{CHCl}_3$  solns depend upon the Pb range it is desired to cover and are so chosen as to give same general color progression from red to green for each range. Limiting the range increases accuracy at expense of flexibility. Colors produced with standard amounts of Pb furnish by comparison the basis for a quantitative estimation. Volumes and concentrations of standard dithizone for various ranges are as follows:

Pb RANGES micrograms (0.001 mg)	CONCENTRATION mg/liter	VOLUME ml	CELL LENGTH mm
0–5	4	5	50
0–10	4	10	50
0–20	8	10	25
0–50	8	25	25
0–100	10	30	12.5
0–200	20	30	12.5

(a) *Simple Color Matching*.—Prepare 10 standards covering in equal steps the range in which it is desired to work, as follows: Use a standard Pb soln, 29.36(a), in 1%  $\text{HNO}_3$ , 1 ml of which equals some simple fraction or multiple of 1 microgram of Pb. Measure the amounts representing the various steps of the range into a series of separators and add the pure 1%  $\text{HNO}_3$  so that total volume is always 50 ml. (It is best to add the acid first so that the Pb soln is not lost around the stopcock of the separator.) Add 10 ml of the ammonia-cyanide mixture, 29.36(j), and mix. Resultant pH will be ca 9.7. Immediately add the appropriate volume of standard dithizone, which depends upon range to be covered (see table), and shake for 1 min. Draw off lower layers into series of tubes or vials and arrange in order. For lower ranges, i.e., up to 20 micrograms of Pb, matching is best done by viewing longitudinally in small flat-bottomed vials ca 3" in length. For higher ranges, 0–50 micrograms and above, depth of column must be reduced, and matching is conveniently done by viewing transversely in Nessler tubes of matched diameter, because even pure dithizone solns appear red by transmitted light if concentration or depth of column is increased beyond a certain point. If standards are kept covered when not in use they should last at least one day.

For the determination, place aliquot part, or entire amount, of the 50 ml of 1%  $\text{HNO}_3$  in which the Pb has been isolated, 29.39(b) or 29.40(b), in a separator, and if aliquot is taken, make to 50 ml with the 1%  $\text{HNO}_3$ . Add 10 ml of the ammonia-cyanide mixture, 29.36(j), and mix. Immediately develop the color by shaking 1 min. with proper amount of the standard dithizone. Draw off lower layer into tube or vial similar to those used with standards and compare. If range is exceeded, repeat with smaller aliquot, or re-extract with excess dithizone before draining from separator, isolate once more in 50 ml of the  $\text{HNO}_3$  reagent, and compare with stand-

ards covering a higher range. Interpolation between steps of the various ranges should be easily made. If an aliquot of the 50 ml of the 1%  $\text{HNO}_3$  in which the Pb has been isolated is taken, subtract only a corresponding amount of the total reagent blank from amount of Pb found.

(b) *Photometric Methods*.—Transmission spectra of the two components in the dithizone extract (Pb dithizone complex and the free dithizone) show a marked difference in their ability to absorb light of wave length 510  $\mu$ , the red Pb complex absorbing strongly and the green dithizone transmitting freely. Thus, when the absorption of light of this wave length by the individuals of a standard color series, measured thru suitable cell-length, is determined photometrically, a linear relation is observed between amounts of Pb and absorbency ( $-\log$  transmittancy). In making the measurements a spectrophotometer set at this wave length or a simple photometer equipped with a blue-green filter centered at about this point can be used. The dithizone solns are standardized once only with known amounts of Pb, and the labor of repeated standard preparation is necessary only when changes caused by evaporation or oxidation occur.

Standardize dithizone solns as follows: Using appropriate volumes and concentrations of solns specified for various ranges (see above) in separators, prepare standard colors as in the visual color-matching procedure, saturating the standard Pb and the 1%  $\text{HNO}_3$  solns with clear  $\text{CHCl}_3$  before use, and thereby eliminating differences in volume of extract between standards and unknowns. (It is unnecessary to prepare the full 10 steps of the range, and the number of standards may be limited to 5 or 6.) Develop the colors by shaking the separators 1 min., allow to stand a few minutes, and filter extracts thru specially prepared filter papers (9 cm quantitative filters soaked overnight in 1%  $\text{HNO}_3$  and washed with large volumes of  $\text{H}_2\text{O}$  on Büchner funnel to remove the slight trace of acid and/or Pb usually present on even the best grades of filter paper. Fitting a 9 cm filter directly into mouth of a 50 ml low-form Pyrex beaker eliminates the need of a funnel in the filtering operation). Fill a cell of proper length with the filtered extracts for the various Pb ranges, using the specified volume and strength of standard dithizone solns. Cell lengths appropriate for photometric instruments covering an absorbency range of 0–2 (100–1 per cent transmittancy), such as the Bausch and Lomb spectrophotometer or the neutral wedge photometer (20), are given for the various ranges in the preceding table. If instruments which do not cover so large a transmittancy range are employed, shorter cells must be used.

Determine *absorbencies* for the various steps of the range and plot against the quantity of Pb to obtain a standardization curve for the particular lot of dithizone. Preferably calculate slope of line connecting the standard points and the intercept of the line on the Pb axis, making calculation as follows: Take equation of line connecting standard points as  $X = a + bY$ , and let  $X$  = micrograms of Pb and  $Y$  = absorbency;  $a$  then represents intercept on Pb axis (in this case a negative value) and  $b$  represents tangent or slope of line. Calculate  $a$  and  $b$  from following formula, where  $n$  = No. of observations, including that for 0 Pb, and  $\Sigma$  represents merely "the sum":

$$b = \frac{\Sigma XY - \frac{\Sigma X \Sigma Y}{n}}{\Sigma Y^2 - \frac{\Sigma Y \Sigma Y}{n}}, \text{ and } a = \frac{\Sigma X}{n} - b \frac{\Sigma Y}{n}.$$

Then the procedure for determining the Pb content of an unknown falling within

the range is to determine absorbency, using the standard dithizone and the same cell with which standard readings were made, and calculate the Pb from the equation,  $X = a + bY$ , using values of  $a$  and  $b$  determined above. If protected from evaporation and direct sunlight, standard factors of dithizone solns should not change appreciably for at least a month (15).

For the actual determination proceed as directed in (a), except to filter the extract before photometric measurement thru the prepared filter papers. Determine absorbency, using the standardized dithizone with same cell used in making standard curve, and read amount of Pb from this standard curve or calculate from factor of dithizone soln. If the range is exceeded, repeat with smaller aliquot, or re-extract and repeat with dithizone standardized to cover a higher range. If an aliquot of the 50 ml of 1%  $\text{HNO}_3$  in which the Pb has been isolated is taken, subtract only a corresponding amount of total reagent blank from amount of Pb found.

### 29.43

#### INTERFERENCES

If present in excessive quantities in the final determination, Cl,  $\text{P}_2\text{O}_5$ , As, Se, Te, Hg, and Bi (>5 mg) will prevent the complete electrolytic deposition of Pb; and Bi (<2 mg), Sn, Sb, Mn, and Ag will contaminate the deposit. Certain reducing agents, such as nitrites, likewise prevent complete deposition of the Pb. The general method leading up to the final determination of Pb by the electrolytic procedure has been so formulated that all interferences except those of Sn and Bi are eliminated. Special directions, applicable to both electrolytic and colorimetric methods, for removal of Sn and Bi are given in 29.44 and 29.45. As much as 3 mg of Cl, 20 mg of  $\text{As}_2\text{O}_3$ , 30 mg of  $\text{P}_2\text{O}_5$ , and 50 mg of Hg will not interfere in the final electrolysis, and if there is suspicion that greater quantities are present in the sulfide mixture, 29.40, they can be eliminated by a dithizone extraction. Interferences in the colorimetric dithizone method are limited by the use of KCN to stannous Sn, Bi, and Tl. The rarity of Tl makes its interference unlikely in ordinary work, and no method of removal is given (17). Dithizone itself is destroyed by strong oxidizing agents, such as free halogens, and large quantities of ferric Fe, under conditions of dithizone extraction of Pb.

### 29.44

#### Removal of Tin

Sn becomes a problem in the analysis of canned foods, and in quantities above 150 p.p.m. it will usually appear in the ash soln as a milky suspension of  $\text{SnO}_2$ . It must be dissolved to facilitate filtration and to release occluded Pb. Quantities of Sn of this order may cause trouble by precipitating under the conditions of the dithizone extraction of Pb, 29.39.

Two procedures for elimination of larger quantities of Sn are given: (a) volatilization as  $\text{SnBr}_4$  from acid soln of ash, and (b) leaching the mixed sulfides with warm Na polysulfide, when sulfide method of isolation, 29.40, has been applied. These procedures may not eliminate Sn completely, but quantity should be reduced to below that necessary to interfere with electrolytic determination of Pb. Stannic Sn is not extracted with dithizone, and as small quantities of residual Sn will be in stannic form after application of either (a) or (b), final isolation of Pb by means of a dithizone extraction will result in complete removal of Sn.

In general, quantities of Sn under 100 mg should not interfere in either the electrolytic or colorimetric dithizone methods of Pb determination provided the Sn is in stannic form and a preliminary isolation with dithizone is made; hence, this method of isolation should be applied wherever possible.

(a) *Volatilization as  $\text{SnBr}_4$  from acid soln of ash.*—After a practically C-free ash has been obtained, 29.37, add 15–20 ml of 40% redistilled HBr. If nitrates have been used as ash aids, cover casserole with watch-glass and heat on steam bath until Br evolution diminishes, then rinse off watch-glass with  $\text{H}_2\text{O}$  and bring to boil to complete expulsion of Br. (This process destroys undecomposed nitrates.) Add more HBr, if necessary, to dissolve the ash and examine the solns for clearness. If there is an insoluble residue of  $\text{SnO}_2$ , add 50–100 mg of pure Sn, 29.36(k), to the simmering HBr soln of the ash and allow it to dissolve. (Metallic Sn seems to be the best agent to bring ignited  $\text{SnO}_2$  into soln. To be effective, ash soln must be in reduced state.  $\text{Fe}_2\text{O}_3$  sometimes becomes “noble” during ashing and dissolves with difficulty, but treatment with metallic Sn also brings it into soln. Treatment with Sn will be necessary only with the contents of badly corroded cans.) When soln of the ash is free from milkiness due to  $\text{SnO}_2$ , add 20 ml of 60%  $\text{HClO}_4$  (double distilled preferred), oxidize mixture with a few ml of the HBr- $\text{Br}_2$  mixture, 29.36(l), and then add a further 15 ml of the reagent, portion-wise, while soln is evaporated to incipient fumes of  $\text{HClO}_4$  (ca  $150^\circ$ ) on hot plate. Repeat with another 10 ml portion of the HBr- $\text{Br}_2$  mixture if more than 100 mg of tin has been used to dissolve the ash. (Hot  $\text{HClO}_4$  helps keep the ash salts in soln and with Br holds the Sn in the volatile  $\text{SnBr}_4$  combination.) When the HBr and Br have been completely volatilized, cool, and take up with hot  $\text{H}_2\text{O}$  (200 ml may be necessary if much  $\text{KClO}_4$  is present). Filter off any small quantities of dehydrated  $\text{SiO}_2$ , extract residue twice with 5 ml of the hot HCl-citric acid reagent, 29.36(n), and hot  $\text{H}_2\text{O}$ , treat dish if necessary with NaOH as directed in 29.37(l), and isolate the Pb by dithizone extraction as directed under 29.39, or by sulfide separation, 29.40, finally determining Pb as directed under 29.41(b) and (c) or 29.42(a) and (b).

(b) *With sodium polysulfide.*—(Recommended for routine work on canned foods by the electrolytic method when  $\text{Pb} > 0.05$  mg.)

Isolate the Pb by means of a sulfide precipitation, 29.40, filter, and wash flask and filter with 3–6 portions of ca 5 ml each of the warm Na polysulfide soln, 29.36(m). (Sn, As, and Sb sulfides are dissolved; CuS may be partially dissolved and reprecipitated in the filtrate.) Wash flask and residual sulfides several times with 3%  $\text{Na}_2\text{SO}_4$  soln adjusted to pH 3.0–3.4 and saturated with  $\text{H}_2\text{S}$ , and proceed as directed under 29.40(a), beginning “dissolve sulfides with 5 ml of hot  $\text{HNO}_3$ ,” and continuing directly to electrolytic determination, 29.41(b) and (c). When ash contains much Sn, as when metallic Sn has been added to dissolve insoluble metallic oxides, the sulfide precipitate will be so bulky as to be difficult to handle, and it will be necessary to use the volatilization procedure (a) before sulfiding. For the colorimetric dithizone determination of Pb extract  $\text{HNO}_3$  soln of dissolved sulfides and proceed as directed under 29.40(b), 29.42(a) or (b).

## 29.45

### *Detection and Removal of Bismuth*

(a) *By dithizone at pH 2.0 after preliminary dithizone extraction at pH 8–11 (21).*—(This procedure completely removes small quantities of Bi.)

Extract the metals from the  $\text{CHCl}_3$  dithizone extract with 50 ml of 1%  $\text{HNO}_3$  as directed in 29.39(b). Adjust acid extract to pH 2.0 (metacresol purple indicator) with 5%  $\text{NH}_4\text{OH}$  soln and shake vigorously ca 1 min. with 10 ml of a  $\text{CHCl}_3$  soln of dithizone containing 200–250 mg/liter. Allow layers to separate, and if  $\text{CHCl}_3$  extract is orange red to red (Bi), draw it off and extract with another 10 ml portion of the dithizone soln. If shades of green or purple are visible, indicating excess of dithizone, draw off  $\text{CHCl}_3$  extract and extract aqueous phase once more with 5 ml of the dithi-

zone soln (shaking should be prolonged, 3–5 min., to insure complete extraction of Bi). Continue extractions until dithizone extract remains pure green. Adjust pH of aqueous soln to 8.5 with  $\text{NH}_4\text{OH}$ , add KCN, and extract with dithizone as directed in 29.39. Determine Pb colorimetrically as directed in 29.39 (b), and 29.42 (a) or (b), or electrolytically, 29.39 (a), 29.41 (b) and (c) when the Pb > 0.05 mg.

(Attention is directed to the procedure of Bambach and Burkey (22), who separate small amounts of Bi from Pb by shaking out the  $\text{CHCl}_3$  soln of their mixed dithizonates with an aqueous soln buffered at pH 3.4; the Bi remains as the dithizonate in the  $\text{CHCl}_3$  phase, while the Pb enters the aqueous phase and can be separated Bi-free. Only slight excess of free dithizone should be present in the  $\text{CHCl}_3$  mixture of dithizonates, otherwise Pb does not strip out completely. A system of photometric detection and evaluation of Bi interference has also been outlined (17).)

(b) *From acid soln of sulfides*.—(Intended for small quantities of Bi, particularly when sulfide separations may be necessary.) Dissolve mixed sulfides, 29.40, with hot  $\text{HNO}_3$  and separate Bi and Pb as directed in (a).

*Special conditions*.—(Intended for products containing large quantities of Bi.) Dissolve inorganic Bi compounds directly in  $\text{HBr-Br}_2$ , 29.36(1). Prepare organic Bi compounds or Bi preparations mixed with organic matter containing little ash, as directed in 29.37, and dissolve residue in  $\text{HBr-Br}_2$ . If sample contains organic matter with appreciable ash material other than Bi compounds, proceed as directed in 29.37 or 29.47, apply sulfide separation, 29.40, and dissolve mixed sulfides in  $\text{HNO}_3$ . Evaporate  $\text{HNO}_3$  soln of sulfides to dryness in porcelain dish and treat with small portions of  $\text{HBr-Br}_2$  mixture. Evaporate contents of dish containing Bi dissolved in  $\text{HBr-Br}_2$ , after any of above methods of preparation, on steam bath to volatilize Sn and to convert other metals to bromides. Evaporate to dryness and place in temp.-controlled muffle and raise temp. gradually to  $300^\circ$ . ( $\text{AsBr}_3$  and  $\text{SbBr}_3$  will volatilize first at  $100^\circ$  or above;  $\text{BiBr}_3$  will volatilize in dense orange fumes when temp. reaches  $300^\circ$ .) At end of 5 min. or when fumes are no longer evolved, remove dish, cool, and treat again with small portions of  $\text{HBr-Br}_2$ . Again evaporate to dryness and heat for an additional period of 5 min. at  $300\text{--}325^\circ$  ( $\text{PbBr}_2$  does not volatilize appreciably below  $350^\circ$ ). Remove dish, cool, and dissolve residue in hot  $\text{HNO}_3$ . Proceed with removal of last traces of Bi at pH 2.0 and determine Pb as directed in (a).

(c) *After  $\text{PbO}_2$  titration in electrolytic method*.—Add to the soln from 29.41(c) in the titrating vial 0.25 g of solid KI and ca 0.5 ml of HCl. Shake, and add only sufficient  $\text{Na}_2\text{S}_2\text{O}_5$  soln to discharge any starch I color. Pure yellow color shows presence of the double Bi iodide. (Under conditions of test, there is no interfering Cu, ferric Fe, or Sb, and 0.005 mg of Bi will show the yellow color test.) If test is positive, reject Pb results and repeat determination, giving special attention to removal of the Bi interference.

#### SPECIAL METHODS OF SAMPLE PREPARATION

##### 29.46

##### *Solution in Acids*

(Applicable to chemicals soluble in  $\text{H}_2\text{O}$  or acid, e.g., phosphates, sulfates, etc., and organic products of type of tartrates and citrates)

Dissolve 5–100 g of sample in HCl in 400 ml beaker, gaging amount of sample according to its nature and amount of Pb expected. With Ca phosphates use 10–50 g. Dissolve in smallest practicable volume of soln by warming and adding alternately small quantities of hot  $\text{H}_2\text{O}$  and HCl. Filter soln with suction (fritted glass

filter preferred) into beaker or flask under bell jar and leach any residue with 10–25 ml of the hot HCl-citric acid, 29.36(n), followed by 10–25 ml of hot 40%  $\text{NH}_4$  acetate soln. Rinse beaker and filter with hot  $\text{H}_2\text{O}$  and cool soln.

Proceed as directed under 29.39. If interference by precipitate formation occurs, reacidify and isolate Pb by the sulfide precipitation, 29.40. If difficulty is experienced in obtaining clear soln with Ca phosphates at pH 3.0–3.4 (sulfide precipitate may be contaminated with excessive phosphates), redissolve precipitate, add more citric acid soln, 29.36(d), readjust pH, and reprecipitate the sulfides; or make one sulfide precipitation, dissolve the sulfides in hot  $\text{HNO}_3$ , boil off  $\text{H}_2\text{S}$ , and extract Pb with dithizone, 29.39. Sometimes difficulty due to precipitate formation in 29.39 can be obviated by use of smaller sample for extraction and colorimetric determination. If Sn or Bi is suspected, remove by methods described under 29.44 and 29.45. Finally determine the isolated Pb electrolytically, 29.41, or colorimetrically, 29.42.

#### 29.47

#### *Complete Digestion*

(Applicable to most food or biological products; with difficulty to fats and oils, oily products, etc.)

Digest a representative sample in Kjeldahl flask as directed under 29.3. Distil the As if desired according to bromate method, 29.9. If the As is not to be distilled, add 100 ml of  $\text{H}_2\text{O}$  and sufficient HCl to residue in flask to dissolve any  $\text{CaSO}_4$  that may be present. Filter on fritted glass filter, pulverizing any insoluble residue with flattened stirring rod (anhydrous  $\text{SiO}_2$  or  $\text{BaSO}_4$ ). Dissolve any  $\text{PbSO}_4$  in flask and leach residue on filter with 10–20 ml of the hot HCl-citric acid soln, 29.36(n), followed by 10–20 ml of hot 40%  $\text{NH}_4$  acetate soln. Finally rinse both flask and filter with hot  $\text{H}_2\text{O}$ . Isolate the Pb by dithizone, 29.39, or sulfide precipitation, 29.40, methods. (In general, sulfide method is preferable, especially when  $\text{BaSO}_4$  or excessive  $\text{CaSO}_4$  is present, as insoluble sulfates readily occlude Pb.) If Bi and Sn are present, remove them as directed in 29.44 and 29.45. After isolation determine Pb according to electrolytic, 29.41, or colorimetric method, 29.42.

#### 29.48

#### *Partial Digestion or "Mush"*

(a) *For fruits or vegetables that can be peeled.*—Weigh and peel representative sample (10–45 apples), including if desired, stem and calyx ends with peels. Transfer the peels to one or more 2000 ml tared beakers, re-weigh, and record weight of peels. Add 75–200 ml of  $\text{HNO}_3$  to each of beakers, according to weight of peel therein, and warm carefully over gauze or on steam bath in fume hood. Stew slowly, while stirring, until initial foaming decreases. Cover beaker with watch-glass and continue heating until smooth mixture results with little or no stringiness and greatly diminished evolution of oxides of N (15–45 min. according to amount of sample taken). Colloids (pectin) must be sufficiently destroyed to prevent emulsification in subsequent  $\text{CHCl}_3$  extractions. Dilute with  $\text{H}_2\text{O}$ , cool, and transfer contents of the one or more beakers to 1000 or 2000 ml volumetric flask. Make to mark, mix well, and filter. Transfer 100–250 ml of filtrate to short-stemmed separator, add citric acid soln, 29.36(d), equivalent to 5 g of citric acid, make ammoniacal (soln will darken materially), and proceed with dithizone extraction as directed in 29.39. (If soln contains much sugar, extra cyanide may be necessary and Pb should be extracted immediately. Sugar residues combine with cyanides and weaken or completely destroy "masking" effect of cyanide. If cyanide is combined in ineffective combinations, other metals, notably Zn, may be extracted.) Determine the ex-



tracted Pb electrolytically, 29.39(a), 29.41(b) and (c). Correct for volume occupied by insoluble matter by allowing 0.075 ml/g of peel.

(b) *For products other than fruit and vegetable peels.*—(For carbohydrate foods, fresh or canned small fruits or vegetables, jams, apple butter, etc. Sn is often present, while Bi is usually absent.)

Weigh 100–200 g of well-mixed sample into 1000–2000 ml beaker. To dry samples, add about an equal weight of H<sub>2</sub>O, add 50–150 ml of HNO<sub>3</sub> and “mush” mixture as directed in (a). (Duration of mushing period and quantity of HNO<sub>3</sub> should be varied according to the product. Colloids, which induce emulsification in the dithizone extraction, should be destroyed so that clear soln is obtained upon filtration.) Cool, transfer to 500 ml flask, mix well, and filter. Transfer 100–250 ml aliquot of filtrate to separator and proceed as directed in (a), concluding with electrolytic determination. (Interference of Sn is generally negligible.)

## RAPID METHOD RESTRICTED TO APPLES AND PEARS—OFFICIAL

(Efficiency of 95% expected)

(For rapid determination of Pb spray residue on apples and pears. (P.p.m.  $\times$  .007 = grains/lb.))

### 29.49

#### PREPARATION OF SAMPLE

Weigh 10 or more apples or pears and pull or cut out stems with narrow-bladed knife, cutting no more of flesh than necessary. Trim off sepals (dried residue of blossom) and discard sepals and stems. To 25 ml of 30% NaOH soln in 600 ml beaker, add 175 ml of H<sub>2</sub>O and 25 ml of Na oleate soln, 29.36(o), and bring to gentle boil. Have ready in wash bottle 250 ml of hot HNO<sub>3</sub> (2+98) or hot HCl (3+97). (A reasonably accurate figure for As<sub>2</sub>O<sub>3</sub> can be obtained by employing the HCl rinse, and applying a Gutzeit As determination, 29.5, to portion of the filtrate, after acidifying part of the 500 ml of alkaline strip soln with one-tenth volume of HCl instead of HNO<sub>3</sub> (see later in this paragraph). The rapid tentative method for F, 29.32, likewise employs an HCl rinse and acidification.) Impale each fruit in turn upon pointed glass rod, immerse in the alkaline soln, with occasional rotation until skin begins to check, then remove to large funnel inserted in 500 ml volumetric flask and rinse with stream of the hot acid, being careful to flush out stem and calyx ends thoroly. When all fruit has been thus treated, cool alkaline soln and add it thru funnel to acid soln in flask. Rinse beaker and funnel with any remaining acid and with H<sub>2</sub>O, using entire 250 ml of rinse acid. Cool, and make to volume. In dry 200 ml Erlenmeyer flask place exactly 10 ml of HNO<sub>3</sub>. Thoroly mix contents of volumetric flask and immediately add 100 ml to acid in Erlenmeyer flask while swirling vigorously. Filter on rapid filter. If first portion of filtrate is cloudy, return it to filter until clear filtrate is obtained. Determine Pb as directed in 29.50 or 29.51, or use 25 ml of acid and 250 ml of wash soln and proceed electrolytically as directed under 29.52.

#### DETERMINATION

### 29.50

#### *With Nessler Tubes*

(At least 15 tall-form tubes matched for uniformity in color and diameter are necessary)

(a) *Standards.*—Introduce into each of two 1 liter volumetric flasks 47.5 ml of 30% NaOH soln. When HNO<sub>3</sub> has been used in rinsing and acidification, 29.49, add

100 ml of  $\text{HNO}_3$  to each flask. When  $\text{HCl}$  (3+97) has been used in rinsing, add 91 ml of  $\text{HNO}_3$  and 13.6 ml of  $\text{HCl}$  to each flask. Do not mix in the acids unless solns are cold and dilute. To one of flasks add stock reagent, 29.36(a), equivalent to 25.45 mg of Pb. Mark this flask "standard" and the other "blank." Dilute both solns to volume at room temp. and mix. These two solns contain the reagents as they occur in an acidified and filtered sample soln. The "standard" is equivalent in Pb content to an acidified soln from a sample of 1400 g carrying a Pb load (removable by "stripping" procedure) of 10 p.p.m. By a combination of the two solns in suitable proportions the equivalent of any Pb load from 0 to 10 p.p.m. may be obtained.

The standard tubes may be made up in intervals corresponding to 1.0 p.p.m. and then interpolation to 0.5 p.p.m. is possible. Following table gives the quantities of "standard" and "blank" to be added to the Nessler tubes for each interval; they are conveniently measured into the tube by means of burets.

p.p.m.	"STANDARD" ml	"BLANK" ml
0.0	0.0	10.0
1.0	1.0	9.0
2.0	2.0	8.0
3.0	3.0	7.0
4.0	4.0	6.0
5.0	5.0	5.0
6.0	6.0	4.0
7.0	7.0	3.0
8.0	8.0	2.0
9.0	9.0	1.0
10.0	10.0	0.0

Working with one tube at a time add to each tube 10 ml of the ammonia-cyanide-citrate soln, 29.36(p), followed by 30 ml of standard dithizone soln (30 mg of purified dithizone dissolved in 1 liter of  $\text{CHCl}_3$  and preserved in a dispensing apparatus to prevent evaporation). Shake vigorously for 1 min. and allow layers to separate. The pH of the aqueous phase should be ca 9.4 regardless of whether  $\text{HCl}$  or  $\text{HNO}_3$  is used in rinsing. Stopper each standard tube securely with new cork stopper. It is unnecessary to make up entire series of standards if only a portion of the range, for example 5.0–10.0 p.p.m., is of quantitative interest.

(b) *Comparison*.—Transfer 10 ml portions of the clear filtrate from 29.49 to each of three Nessler tubes. First add 10 ml of the ammonia-cyanide-citrate soln, 29.36(p), to each tube; to one tube add 30 ml of standard dithizone soln, (a), and to the other two tubes 30 ml of clear  $\text{CHCl}_3$ . Shake tubes vigorously for 1 min. and allow layers to separate. With a tube of clear  $\text{CHCl}_3$  backing the sample tube (containing the dithizone) and one sample tube containing  $\text{CHCl}_3$  backing each of two standard tubes, compare the color in the lower layer of the sample with that of the standards, looking thru tubes at right angles to their lengths toward a strong diffused light. (A comparator box similar to the boxes used in colorimetric pH measurements but of larger size will be found convenient. When working with apple strip solns, a slight turbidity is produced in the sample tube, which slightly changes the color observed. To compensate for this effect, the same turbidity is introduced in the field of view of the standard tubes made up exactly as in the sample, except that  $\text{CHCl}_3$  is substituted for the dithizone soln.)

If the range is exceeded, i.e., if the color produced by the sample is redder than the 10 p.p.m. standard, repeat with smaller aliquot of filtrate, making up to 10 ml with the "blank" soln. If, for example, a 5 ml aliquot is taken, the indicated reading must be doubled. After a match has been obtained, calculate result to basis of 10 ml aliquot and 1400 g sample.

## 29.51

*With Photometer*

This procedure lends itself readily to photometric methods of measuring the "mixed color," 29.42(b). Changes in 29.50 are introduced here to prevent formation of colors too dense for measurements. Use 5 ml instead of 10 ml aliquots of the acidified wash soln, 29.49.

(a) *Standards*.—Measure following proportions of "standard" and "blank" solns, 29.50, into separators:

P.p.m.	0.0	2.0	4.0	6.0	8.0	10.0
Standard (ml)	0.0	1.0	2.0	3.0	4.0	5.0
Blank (ml)	10.0	9.0	8.0	7.0	6.0	5.0

Add 10 ml of the ammonia-cyanide-citrate soln, 29.36(p), and working with one separator at a time, immediately develop the colors by shaking 1 min. with 25 ml of pure dithizone soln of 15 mg/liter strength. Allow to stand a few minutes to cool, filter  $\text{CHCl}_3$  layers thru specially washed filter papers, 29.42(b), and fill cell of appropriate length (10 mm for an instrument covering a 0–2 absorbency range, 29.42(b)). Determine absorbencies and plot against p.p.m. of Pb to obtain a standard curve.

(b) *Comparison*.—Place appropriate sized aliquot of acidified strip soln in separator and make up to 10 ml with the "blank" soln. Add 10 ml of the ammonia reagent, 29.36(p), and shake out with 25 ml of the standard dithizone soln. Allow to stand a few minutes to cool, filter, and read as directed above. Determine quantity of Pb from the standard curve prepared as in (a) and calculate to basis of a 5 ml aliquot and 1400 g sample.

## 29.52

## ELECTROLYTIC DETERMINATION OF LEAD IN APPLE FILTRATE

Transfer 200 ml of the acid filtrate to a separator, add equivalent of 5 g of citric acid, 29.36(d), make ammoniacal, add 5 ml of the 10% KCN soln, extract with dithizone as directed in 29.39(a), and finally determine the Pb electrolytically as directed in 29.41(b) and (c).

## 29.53

## MANGANESE—TENTATIVE.—See 12.15, or 26.21

## MERCURY (29)—TENTATIVE

(Applicable to leafy vegetables)

## 29.54

## REAGENTS

(a) *Hydrogen peroxide*.—30% electrolytic soln.

(b) *Standard mercury solns*.—(1) Dissolve 500 mg of pure metal in  $\text{HNO}_3$  and dilute to 1 liter; (2) dilute 10 ml of soln (1) to 500 ml with  $\text{H}_2\text{O}$  containing a few ml of  $\text{HNO}_3$  (1 ml = 0.01 mg of Hg).

(c) *Nitric acid*.—If 50 ml of acid shows a blank of more than 4 micrograms purify by distilling over  $\text{H}_2\text{SO}_4$  in an all-glass apparatus and adjust to standard strength.

(d) *Diphenylthiocarbazone (dithizone)*.—Purify as directed under 29.36(e), if necessary. Prepare as follows:

(1) *Strong soln*.—Dissolve 50 mg of dithizone in  $\text{CHCl}_3$  and dilute to 100 ml.

(2) *Extraction soln*.—Dissolve 10 mg of dithizone in  $\text{CCl}_4$  and dilute to 200 ml.

(3) *Titration soln*.—Measure 10.0 ml of soln (1) into a 500 ml volumetric flask and dilute to mark with  $\text{CHCl}_3$ .

Preserve solns in dark bottles at 5–10°.

## 29.55

## APPARATUS

*Digestion flask and internal condenser.*—Use 2 liter Florence flask of resistant glass, preferably Pyrex, fitted with internal condenser, a glass cylinder ca 9.5" long and ca 1.5" in diam., enlarged at top to retain it in neck of flask. Bottom is cone-shaped and closed. Top may be closed with 2-holed stopper or entire condenser may be made of glass. Condenser has inlet tube extending nearly to bottom, and an outlet tube. Condenser should fit closely inside neck of Florence flask and should extend ca 1.5" into body of flask. Dimensions of condenser will vary slightly according to dimensions of flask.

## 29.56

## DETERMINATION

Introduce into digestion flask suitable weighed portion of the finely chopped and well-mixed sample (100–150 g in case of lettuce). Add 50 ml of  $\text{HNO}_3$  and 200 ml of  $\text{H}_2\text{O}$ , place internal condenser in flask, and start  $\text{H}_2\text{O}$  flowing thru it.

Heat flask over low flame or on hot plate until contents boil, and reflux for 25 min. (In all refluxing, boiling must be gentle, so that top half of neck of flask remains cool.) Remove flask from flame and cool nearly to room temp. Rinse condenser with  $\text{H}_2\text{O}$  from wash bottle and remove it. Filter contents quite rapidly thru large Büchner funnel (11–18.5 cm in diam.). Wash flask with ca 30 ml of  $\text{H}_2\text{O}$ , decanting onto filter when nearly all soln has passed thru. Repeat washing once or twice. Return liquid to digestion flask, washing in with small quantity of  $\text{H}_2\text{O}$  from wash bottle.

Digest as follows: Add 12–14 g of  $\text{KMnO}_4$ . (Add in several portions to cabbage or other foods that react vigorously or froth unduly; to lettuce extract it may be added at once.) Replace internal condenser, and when reaction subsides heat gently to boiling and reflux 10–15 min. Partially cool flask in bath, raise condenser, and add 10–12 g of  $\text{KMnO}_4$  as fast as vigor of reaction will permit.

Replace condenser and again heat soln to boiling 12 min. unless it clears in less time. Cool, add 9 or 10 g of  $\text{KMnO}_4$  and ca 20 ml of  $\text{HNO}_3$ , and repeat digestion and refluxing ca 15 min. Continue cooling, addition of  $\text{KMnO}_4$ , and subsequent heating until purple color of  $\text{KMnO}_4$  persists when liquid is heated almost to boiling. Agitate soln during heating to avoid bumping caused by accumulation of black oxides of Mn. If several more additions of  $\text{KMnO}_4$  are required, add more  $\text{HNO}_3$ . (For each 4 g of  $\text{KMnO}_4$  used 5 ml of  $\text{HNO}_3$  is required to combine with the K and reduced Mn. All but the most refractory organic matter should be oxidized, otherwise nitrites, formed by the action of  $\text{HNO}_3$  on organic matter, will decompose the dithizone, as shown by loss of color, and thus prevent complete extraction of Hg. Oxidation is sufficiently complete when supernatant liquid appears colorless after the  $\text{MnO}_2$  has settled out.)

Cool, and add  $\text{H}_2\text{O}_2$ , a little at a time, while shaking with rotary motion, until precipitated oxides of Mn dissolve completely. Be careful to insure an excess of  $\text{HNO}_3$ , otherwise a large quantity of peroxide may be used to no advantage. Replace condenser and heat to boiling 8 min. to remove free O and to dissolve refractory particles, then cool again. Add 0.5 g of crystallized hydroxylamine sulfate or chloride (soln should have strength of ca 0.5%  $\text{HNO}_3$ ). Conduct a blank determination on the reagent used. One blank may be used for a particular batch of acid or  $\text{KMnO}_4$ , etc.

To concentrate the Hg and remove interfering substances, extract liquid in portions not to exceed 425 ml as follows:

Add 2 ml of dithizone soln (2), shake vigorously ca 15 seconds, allow the  $\text{CCl}_4$  phase to separate, and note its color. (The Hg-dithizone complex is orange yellow.

The observation will be used in the determination later.) Add an additional 6 ml of the dithizone reagent (2), shake, etc., and again note color of  $\text{CCl}_4$  layer. Then add 10 ml of the reagent, shake vigorously 20–30 seconds, and after separation of liquids carefully draw off extract into clean separator (125–250 ml). If extract is still orange yellow, continue the extraction with 20 ml portions of reagent as long as the orange yellow extract is obtained. Remove extract from separator containing the first extract each time. (The bright orange-yellow color of the Hg complex should not be confused with the slow fading of the dithizone reagent to the weak green or yellow that sometimes occurs due to oxidation.) When a green, light green, or reddish extract is obtained, extract once more with a combination of 5 ml of dithizone soln (2) and 10 ml of plain  $\text{CCl}_4$ . (The red or reddish violet color is due to Cu that is extracted after the Hg and continuing the extraction will only remove more Cu.) If the last extractions are red (Cu), add to combined extracts 20 ml of dithizone soln (2) to assist in inhibiting the transfer of Cu with  $\text{Na}_2\text{S}_2\text{O}_3$ .

To the combined dithizone- $\text{CCl}_4$  extracts, etc., add 40 ml of  $\text{H}_2\text{O}$ , 1 ml of  $\text{HNO}_3$  (1+19), and 5 ml of 1%  $\text{Na}_2\text{S}_2\text{O}_3$  soln. Close separator and shake vigorously 30 seconds. Allow layers to separate and draw off  $\text{CCl}_4$  layer. Filter aqueous layer thru plug of wet cotton in short-stemmed funnel into 500 ml Erlenmeyer flask to remove any  $\text{CCl}_4$  droplets. Wash separator with two 5 ml portions of  $\text{H}_2\text{O}$  from a wash bottle and then use these portions to wash the short-stemmed funnel by allowing the  $\text{H}_2\text{O}$  to run down sides of funnel. Add 1 ml of  $\text{HNO}_3$  (1+1) and 6 ml of saturated  $\text{KMnO}_4$  soln to the liquid in the flask. Place rubber stopper (previously boiled in  $\text{H}_2\text{O}$ ) carrying an air condenser in flask and place it on steam bath 8–10 min.

Remove flask from steam bath and cool approximately to body temp. (33–40°). Add slowly (dropwise) 10%  $\text{NH}_2\text{OH} \cdot \text{HCl}$  soln while shaking with gentle rotation until soln clears. Add last few drops very slowly and rotate flask to dissolve any particles on side. Then add 0.4–0.5 ml of the  $\text{NH}_2\text{OH} \cdot \text{HCl}$  soln in excess. Place thermometer in flask and warm on steam bath to 60°. Remove flask and thermometer from bath, and cover flask for 1 min., then cool under cold running  $\text{H}_2\text{O}$ .

If quantity of Hg is greater than 250 micrograms (i.e., when more than the first 8 ml of the previous extracts were orange yellow), make soln to an exact volume and use aliquot for determination; otherwise use entire sample. Place soln to be titrated, at 22–26°, in 125 ml pear-shaped separator. Dilute to 100 ml (mark separator), add 0.45 ml of clear  $\text{CHCl}_3$  (from 1 ml graduated pipet or buret graduated to 0.05 ml), and shake to saturate aqueous layer with  $\text{CHCl}_3$ .

*Minute quantities* (0–15 micrograms—i.e., first 2 ml of previous dithizone extractions was green to olive, not full orange yellow.)—Add 1 ml of dithizone (10 mg/liter in  $\text{CHCl}_3$ ) and shake vigorously 20–30 seconds. When layers separate, observe color of  $\text{CHCl}_3$  layer, and if yellow, add another portion of dithizone and shake again (allow all dithizone added to remain in separator), continuing until an olive color indicating an excess of dithizone is obtained (not more than 5–6 ml should be required). Blue-green extract in beginning indicates little or no Hg. Read accurately the dithizone used. In a second separator of same size and shape as first, place 100 ml of an aqueous soln containing 1 ml of  $\text{HNO}_3$  (1+1) and 0.4 ml of 10%  $\text{NH}_2\text{OH} \cdot \text{HCl}$ . Add 0.45 ml of  $\text{CHCl}_3$ , and from the titration required for sample estimate approximate quantity of Hg it contains (4 micrograms/ml will turn the dithizone yellow). Add the estimated quantity of standard Hg soln to second separator. Titrate with dithizone as in sample until colors of extracts are the same, using white background to observe them. If colors show considerable hue differences, it is probably due to incomplete elimination of Cu. The Cu should then be removed by the procedure given under *Removal of Copper*. (Titration is pref-

erably done by adding to the standard a smaller quantity of dithizone than is required by the Hg present to obtain a yellow extract and then adding more dithizone until colors are alike.) From the titrations and the Hg in the standard, calculate Hg in sample as follows:

$$\frac{A}{S} \times \text{micrograms Hg in standard} = \text{micrograms Hg in sample titrated.}$$

$A$  = ml dithizone added to sample, and  $S$  = ml dithizone added to standard.

**Large quantities** (16–250 micrograms).—Add the standard dithizone soln (10 mg/liter) in 3 ml portions, shaking after each addition. Draw off extracts after each second addition if it is saturated with Hg (full orange yellow). Continue to add the dithizone soln until a dirty yellowish green extract (as compared with the saturated Hg complex) is obtained. Add sufficient standard Hg soln to produce the full orange-yellow saturated Hg-dithizone complex and add 8–10 micrograms in excess.

Measure accurately Hg soln added. Shake separator vigorously 20 seconds to saturate the dithizone and after layers have separated draw off lower solvent layer. Filter aqueous portion thru a plug of damp cotton in a short-stemmed funnel into another separator to remove  $\text{CHCl}_3$  globules. Wash once or twice with 5 ml portions of  $\text{H}_2\text{O}$ . Determine the Hg remaining in aqueous soln by the comparative titration for small quantities previously given. Determine Hg equivalent of dithizone when fully saturated with Hg by titrating a standard in similar manner. To obtain correct quantity of Hg in sample, subtract that added at end of titration from total Hg equivalent of the dithizone used.

**Removal of copper.**—Ordinarily Cu is removed by the  $\text{Na}_2\text{S}_2\text{O}_3$  transfer, but if the sample is not rendered sufficiently free by this procedure remove as follows: Shake combined dithizone extracts with 60 ml of  $\text{H}_2\text{O}$  containing a few drops of  $\text{H}_2\text{SO}_4$  (1+1), a few crystals of KI, and a few drops of 5%  $\text{NaAsO}_2$  soln to prevent the liberation of free I. Shake vigorously ca 20 seconds and carefully draw off dithizone layer. Wash aqueous soln with a little  $\text{CHCl}_3$ . (This treatment leaves the Cu in the extract and transfers the Hg to the aqueous phase.) Make solns slightly ammoniacal and extract with dithizone until an orange-yellow extract is no longer obtained. Transfer the Hg to an aqueous soln with acid  $\text{Na}_2\text{S}_2\text{O}_3$  soln as previously directed and continue determination.

#### SELENIUM (24)—OFFICIAL

##### 29.57

##### PRINCIPLES

The procedure consists of wet digestion with  $\text{HNO}_3$  and  $\text{H}_2\text{SO}_4$  in presence of  $\text{HgO}$  fixative; separation of Se by distillation as the volatile bromide; reduction of the bromide to elementary Se with  $\text{SO}_2$ ; isolation and estimation as  $\text{H}_2\text{SeO}_3$  by titration with standard  $\text{Na}_2\text{S}_2\text{O}_3$  and I.

##### 29.58

##### REAGENTS

(a) *Sulfuric-nitric acid soln.*—To 50 ml of  $\text{H}_2\text{SO}_4$  add 100 ml of  $\text{HNO}_3$ . Cool mixture before using.

(b) *Mercuric oxide fixative.*—Dissolve  $\text{HgO}$  in  $\text{HNO}_3$  in proportion of 5 g/100 ml of the acid.

(c) *Concentrated hydrobromic acid-bromine soln.*—Mix 10 ml of liquid Br with 990 ml of  $\text{HBr}$ . (Reagent-grade  $\text{HBr}$  is offered commercially in two concentrations: the constant boiling mixture of 48% (8.1  $N$ ) and the 40% concn (7  $N$ ). Either

may be used, as determination allows for varying quantity of reagent taken according to concentration of HBr.)

(d) *Dilute hydrobromic acid-bromine soln.*—To 5 ml of HBr add 10 ml of saturated Br-water and dilute to 100 ml with H<sub>2</sub>O.

(e) *Sulfur dioxide.*—The gas supplied in commercial cylinders is free of Se.

(f) *Hydroxylamine hydrochloride soln.*—10% W/V in H<sub>2</sub>O.

(g) *Phenol soln.*—5% W/V in H<sub>2</sub>O.

(h) *Standard sodium thiosulfate soln.*—Prepare from accurately standardized 0.1 N reagent with recently boiled H<sub>2</sub>O. Before adjusting to final volume add 5 ml of amyl alcohol/liter and shake vigorously. 1 ml of 0.001 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> is theoretically equivalent to 19.8 micrograms of Se. (For the estimation of Se in quantities greater than 50–75 micrograms proportionately higher concentrations of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> are required.)

(i) *Standard iodine soln.*—Prepare from 0.1 N reagent. Before final dilution add KI in proportion of 20 g/liter. Dilute to like normality of thiosulfate.

(j) *Standard selenium soln.*—Dissolve 250 mg of C.P. Se in conc. HBr-Br. soln (1 ml liquid Br+25 ml conc. HBr, both of which have been distilled). After complete soln has been effected, *almost* neutralize the excess Br with the SO<sub>2</sub> gas while shaking vigorously. Complete the neutralization by adding the phenol soln dropwise in slight excess. Make to 250 ml with H<sub>2</sub>O. (SO<sub>2</sub> must not be present in excess because it would then reduce the H<sub>2</sub>SeO<sub>3</sub> to the element.) If too much SO<sub>2</sub> has been used, add Br water until the color of the selenite soln is slightly but definitely yellow and then complete the neutralization with the phenol. (Unless the analyst is positive that the Se reagent is pure, he must purify it. To do this dissolve ca 1 g of the Se in an excess of the conc. HBr-Br soln, precipitate with SO<sub>2</sub>, warm on steam bath for 30 min., cool, filter, first wash free of acids with H<sub>2</sub>O and then wash with small portions of alcohol, dry at 100° for 1 hour, and prepare the 1 mg/ml standard as described above. This precaution is necessary since the Se soln serves as the ultimate standard in the determination.) Make appropriate dilutions of the concentrated reagent by adding H<sub>2</sub>O, and do not allow the acidity, determined by titration, to fall below .05 N, since neutral or very slightly acid solns of dilute H<sub>2</sub>SeO<sub>3</sub> tend to oxidize and lose their titer. A dilution of 20 micrograms of Se/ml is convenient for micro determinations, for then it is almost chemically equivalent to accurately prepared 0.001 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (1 ml of 0.001 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> = 19.8 micrograms of Se).

(k) *Soluble starch indicator.*—0.5% W/V.

## 29.59

### APPARATUS

An all-glass distillation outfit consisting of 250 ml round-bottomed flask, still head, thermometer registering to 135°, and condenser with dipping end.

## 29.60

### DETERMINATION

Place 5–10 g (dry weight) of sample in 600–800 ml Pyrex beaker and add 10 ml of the HgO fixative followed by 150 ml of the H<sub>2</sub>SO<sub>4</sub>-HNO<sub>3</sub> soln. Mix thoroly at once and place on steam bath for 30 min., stirring intermittently. If the product is rich in Se, use 1 g of representative material. To dry leafy products such as hop leaves, which oxidize violently, add 25 ml of H<sub>2</sub>O before applying fixative. Heat over burner (not full flame) until digestion mass lightens and then turns brown. Remove flame, cool, and after adding 10 ml of HNO<sub>3</sub>, again heat until first brown appears.

Repeat this operation at least twice and then heat until liquid turns a distinct brown (not black) or until  $\text{SO}_2$  fumes appear. (It is imperative that excess  $\text{HNO}_3$  be expelled and that organic matter be sufficiently oxidized so that the Br reagent subsequently added is not reduced, but prolonged fuming to  $\text{SO}_2$  is to be avoided.) As such products as molasses and honey, principally sugars, react vigorously with  $\text{HNO}_3$ , remove such samples from steam bath until reaction subsides and then proceed in usual manner.

Cool digest and transfer with two 25 ml portions of  $\text{H}_2\text{O}$  to the distilling flask. (If digestion has been carried out in the 250 ml distilling flask, it is necessary even then to add 50 ml of  $\text{H}_2\text{O}$  so that the HBr will distil subsequently as liquid and not as vapor.) Rinse beaker carefully with 25 ml of the HBr-Br soln and add to cooled digest and washings. (If the constant-boiling grade of HBr has not been used, an equivalent volume of less concentrated reagent must be added, e.g., 30 ml of 40% concn, and distillate must have an acidity of ca 2.5 N.) After swirling flask, distil, until the temp. of distillation reaches  $130^\circ$ , into 125 ml Erlenmeyer flask, marked at 50, 75, and 100 ml, containing 5 ml of HBr and surrounded by cold  $\text{H}_2\text{O}$ . During distillation lift tip of condenser out of liquid in flask after all the Br and ca 15 ml of the acid have distilled. (Free Br should distil in beginning, indicating excess of reagent. If this is not the case, stop distillation, cool, and add an additional 10 ml of the HBr-Br soln. This contingency arises only with insufficient digestion of sample.) Rinse condenser tip carefully with two portions of no more than 2 ml each of  $\text{H}_2\text{O}$ . Between analyses rinse condenser tube free of fatty and waxy material with hot  $\text{H}_2\text{O}$  but *do not* add rinsings to distillate. For the next three steps it is assumed that distillate contains no fats, waxes, or other insoluble matter. (1) If volume of distillate and rinsings is 75 ml or less, pass in  $\text{SO}_2$  in excess (ca 30 seconds after complete decolorization of the Br), add 1 ml of the  $\text{NH}_2\text{OH}\cdot\text{HCl}$ , and place mixture on active steam bath for 30 min. Cap flasks with watch-glasses during the various heat treatments. (2) If volume of distillate is between 75 and 100 ml, reduce with  $\text{SO}_2$  and the  $\text{NH}_2\text{OH}\cdot\text{HCl}$  as directed in (1), add several glass beads, bring just to incipient boiling, and complete reduction at once with the 30-min. steam-bath treatment. (3) If volume of distillate should exceed 100 ml, transfer to 200 ml Erlenmeyer flask and complete transfer with four successive 2 ml rinsings with  $\text{H}_2\text{O}$  delivered from pipet. Add 10 ml of HBr, reduce with  $\text{SO}_2$  and  $\text{NH}_2\text{OH}\cdot\text{HCl}$ , add several glass beads, bring to incipient boiling, and place flask at once on steam bath for 30 min. (When volume of soln exceeds 100 ml, recovery of Se may be slightly low.)

If distillate contains fats, waxes, or other insoluble material, filter off with suction on asbestos and rinse receiver flask carefully with four 2 ml portions of  $\text{H}_2\text{O}$  from pipet. Use the successive washings in turn to rinse the asbestos filter. Transfer combined filtrates to 125 ml Erlenmeyer flask (200 ml flask if volume of filtrate exceeds 100 ml), and complete transfer with four 2 ml portions of  $\text{H}_2\text{O}$ , also delivered from pipet. According to final volume of soln, whether less than 75 ml, between 75 and 100 ml, or in excess of 100 ml, proceed with addition of reagents and heat treatment exactly as directed previously. (Se is reduced rapidly from an acid soln 2.5 N or more; steps 2 and 3 are necessary because the acidity is less than 2.5 N in these instances.)

For the assay of products of high Se content (more than 1000 micrograms in the amount of material analyzed) use 50 ml of the HBr-Br soln (or its equivalent volume of a less concentrated grade) for the initial distillation. To such samples add 75 ml of  $\text{H}_2\text{O}$  during the rinsing instead of the usual 50 ml. After the usual distillation, disconnect apparatus and rinse condenser tube with 5 ml of  $\text{H}_2\text{O}$ , which is added directly to distillate. Heat residue in distillation flask to incipient fumes of  $\text{SO}_2$ ,



cool, add 5 ml of  $\text{HClO}_4$ , and heat to fuming. Repeat the  $\text{HClO}_4$  oxidation. (This treatment is necessary for substances like vetches and seedlings, which contain particularly refractory Se compounds.) Cool digest, add two 25 ml portions of  $\text{H}_2\text{O}$  and then 25 ml of the  $\text{HBr-Br}$  soln, and distil to  $130^\circ$  in usual manner. Combine all distillates, and if fats, waxes, or other insoluble matter are present, filter, and wash as previously directed. In either case, adjust to exactly 250 ml in volumetric flask with  $\text{H}_2\text{O}$ , pipet 75 ml into 125 ml Erlenmeyer flask, reduce the Se with excess  $\text{SO}_2$  and  $\text{NH}_4\text{OH.HCl}$ , and complete reduction by heating on steam bath for 30 min.

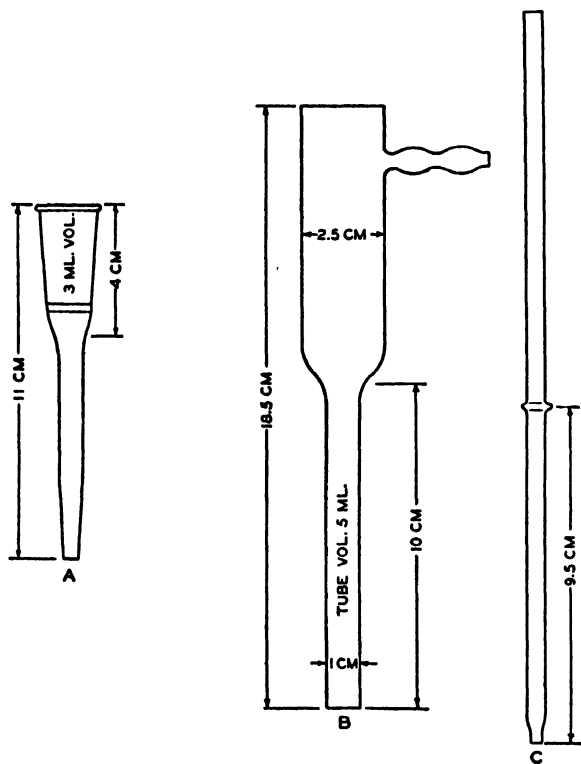


FIG. 47.—FILTRATION AND TITRATION TUBES AND PIPET

Place flasks in cold  $\text{H}_2\text{O}$  (ca  $20^\circ$ ) for a like period and then with suction collect the Se on asbestos pad contained in the filtration vessel, Fig. 47. Rinse precipitation flask and pad with 5 successive 1 ml portions of  $\text{H}_2\text{O}$  from pipet, and then hold mouth of flask before an air vent to remove last traces of  $\text{SO}_2$ .

Insert filtration vessel into titrating tube and dissolve the Se with 1 ml of the dilute  $\text{HBr-Br}$  soln, first adding the reagent from a pipet to the flask and then transferring carefully to the pad. When the Se has dissolved, apply gentle suction and repeat operation with an additional 1 ml of the dilute  $\text{HBr-Br}$  soln. Finally rinse flask and pad with 3 successive 1 ml portions of  $\text{H}_2\text{O}$ , collecting filtrate before each addition; 2 ml of the dilute  $\text{HBr-Br}$  soln is sufficient for Se up to 500 micrograms. When more is present, use proportionately more reagent and rinse  $\text{H}_2\text{O}$ .

Agitate filtrate with pipet stirrer and dispel excess Br with 3 drops of the phenol soln. Using stirrer as a pipet, rinse walls of vessel several times with the soln to neutralize every trace of Br. Immerse titrating tube up to two-thirds of its length in hot  $H_2O$  for 5 min., stirring intermittently. (Heating is required to complete reaction between the Br and phenol.) Then place the vessel in cold  $H_2O$  for at least 5 min. (The Norris-Fay titration (25) works best when soln is below  $25^\circ$ .)

Using original precipitate of Se as a guide, add at least a 50% excess of the appropriate concn of the standard  $Na_2S_2O_3$  soln and 3 drops of the starch indicator. After stirring, add standard I until a permanent blue color appears. If less than 1 ml of I soln is required, add sufficient  $Na_2S_2O_3$  so that at least 1 ml of I is required. Then titrate to colorless end point with  $Na_2S_2O_3$ , adding reagent in increments of .01 ml as end point is approached.

## 29.61

## CALCULATION AND STANDARDIZATION

## (Cross Titration)

To 2 ml of HBr (5+95) contained in the titrating tube add 3 ml of  $H_2O$  and 3 ml of standard I. (The HBr must have been previously distilled.) Titrate with the standard  $Na_2S_2O_3$  soln and toward the end add 3 drops of the starch indicator. Complete titration as directed in 29.60 and obtain  $Na_2S_2O_3$  equivalent of the I.

## 29.62

## STANDARDIZATION OF THIOSULFATE

Add 2 ml of the dilute HBr to an appropriate volume of standard Se soln and after the addition of 50% excess  $Na_2S_2O_3$  soln continue titration exactly as described in 29.61. Obtain the Se equivalent of the  $Na_2S_2O_3$  soln. (As dilute solns of both  $Na_2S_2O_3$  and I always slowly deteriorate, they must be standardized frequently.)

## 29.63

## SAMPLE CALCULATION

The net  $Na_2S_2O_3$  sample titer in ml  $\times$  the Se equivalent = quantity of Se in sample.

## TIN

## 29.64

## PREPARATION OF SAMPLE

Digest a 50–100 g sample as directed under 29.3.

*Gravimetric Method (26)—Tentative*

## 29.65

## REAGENTS

(a) *Wash soln.*—Mix 100 ml of saturated  $NH_4$  acetate soln with 50 ml of acetic acid and 850 ml of  $H_2O$ .

(b) *Ammonium polysulfide soln.*—Pass  $H_2S$  gas into 200 ml of  $NH_4OH$  in bottle immersed in running  $H_2O$  or in ice water until gas is no longer absorbed; add 200 ml of  $NH_4OH$  and dilute with  $H_2O$  to 1000 ml. Digest this soln with 25 g of flowers of S several hours and filter.

## 29.66

## DETERMINATION

Add 200 ml of  $H_2O$  to digested sample and transfer to 600 ml beaker. Rinse the Kjeldahl flask with 3 portions of boiling  $H_2O$ , making total volume of ca 400 ml. Cool, and add  $NH_4OH$  until just alkaline, then 5 ml of HCl or 5 ml of  $H_2SO_4$  (1+3) for each 100 ml of soln. Place beaker, covered, on hot plate; heat to ca  $95^\circ$  and pass in slow stream of  $H_2S$  for 1 hour. Digest at  $95^\circ$  for 1 hour and allow to stand 30 min. longer.

Filter, and wash the precipitate of  $\text{SnS}$  alternately with 3 portions each of the wash soln and hot  $\text{H}_2\text{O}$ . Transfer filter and precipitate to 50 ml beaker, add 10–20 ml of the  $\text{NH}_4$  polysulfide, heat to boiling, and filter. Treat contents of the 50 ml beaker with 2 additional portions of hot  $\text{NH}_4$  polysulfide and wash filter with hot  $\text{H}_2\text{O}$ . Acidify combined filtrate and washings with acetic acid (1+9), digest on hot plate for 1 hour, allow to stand overnight, and filter thru double 11 cm filter. Wash alternately with 2 portions each of the wash soln and hot  $\text{H}_2\text{O}$  and dry thoroly in weighed porcelain crucible. Ignite over Bunsen flame, very gently at first to burn off filter paper and to convert the sulfide to oxide; then partly cover crucible and heat strongly over large Bunsen or Meker burner. ( $\text{SnS}$  must be roasted gently to  $\text{SnO}_2$ , which may be heated to high temp. without loss by volatilization.) Weigh as  $\text{SnO}_2$  and calculate to metallic  $\text{Sn}$ , using factor 0.7877.

*Volumetric Method (27)—Tentative*

## 29.67

## REAGENTS

(a) *Air-free wash soln.*—Dissolve 20 g of  $\text{NaHCO}_3$  in 2 liters of boiled  $\text{H}_2\text{O}$  and add 40 ml of  $\text{HCl}$ . This soln should be freshly prepared.

(b) *Iodine.*—0.01 *N*. Standardize soln frequently against (c), adding asbestos mat and proceeding as directed under 29.68, omitting precipitation with  $\text{H}_2\text{S}$  and the boiling with  $\text{HCl}$  and  $\text{KClO}_3$ . The quantity of  $\text{Sn}$  in the soln used for standardization should equal approximately that contained in sample under examination.

(c) *Standard tin soln.*—Dissolve 1 g of  $\text{Sn}$  in ca 500 ml of  $\text{HCl}$  and dilute to 1 liter with  $\text{H}_2\text{O}$ . 1 ml contains 1 mg of  $\text{Sn}$ .

(d) *Sheet aluminum.*—About 30 gage, free from  $\text{Sn}$ .

## 29.68

## DETERMINATION

Proceed as directed under 29.66 to "Digest at  $95^\circ$  for 1 hour and allow to stand 30 min. longer."

Filter thru asbestos in Caldwell crucible, using suction. Wash the precipitate of  $\text{SnS}$  a few times and transfer detachable bottom and asbestos pad to 300 ml Erlenmeyer flask. Remove all traces of precipitate from inside of crucible by means of a jet of hot  $\text{H}_2\text{O}$  and a rubber-tipped rod, using minimum quantity of  $\text{H}_2\text{O}$  for washing.

Add to flask 100 ml of  $\text{HCl}$  and 0.5 g of  $\text{KClO}_3$ . Boil ca 15 min., making ca 4 more additions of smaller quantities of the  $\text{KClO}_3$  as  $\text{Cl}$  is boiled out of the soln. Wash the particles of  $\text{KClO}_3$  down from neck of flask with  $\text{H}_2\text{O}$  and finally boil to remove  $\text{Cl}$ . Add ca 1 g of the sheet  $\text{Al}$  to dispel last traces of  $\text{Cl}$ .

Fit a 2-holed rubber stopper to flask. Thru one of holes pass bulbed glass tube that reaches nearly to surface of liquid. Attach this tube to large  $\text{CO}_2$  generator thru scrubber containing  $\text{H}_2\text{O}$ . The  $\text{CO}_2$  passes out of flask thru short, bulbed tube inserted in second hole of rubber stopper and terminating slightly below it. Connect this second glass tube by means of rubber tube with another glass tube, ca 10" long, which is immersed in cylinder of  $\text{H}_2\text{O}$  to depth of ca 8". (This connection will act as a seal to restrain any strong flow of gas when not desired and to permit pressure in flask.)

Raise delivery tube nearly out of the  $\text{H}_2\text{O}$  seal, thus allowing rapid flow of  $\text{CO}_2$  for a few minutes to dispel air from system. Then lower delivery tube into  $\text{H}_2\text{O}$  seal, slightly raise stopper, and quickly drop into flask 1–2 g of the  $\text{Al}$  foil, folded into narrow bent strip to prevent breaking flask. When the  $\text{Al}$  has completely dissolved, raise tube in  $\text{H}_2\text{O}$  seal, allowing the  $\text{CO}_2$  to pass thru rapidly; place flask on hot plate and boil for a few minutes. Remove flask from heat and cool with tap

or ice water, continuing flow of  $\text{CO}_2$ . Lower delivery tube into cylinder, disconnect flask, and, with a glass plug, close rubber tube thru which the  $\text{CO}_2$  enters flask. Wash glass tubes, rubber stopper, and sides of flask with the air-free wash soln; add starch indicator, 29.36(h), and titrate immediately with the 0.01 *N* I.

If desired, make the titration by slightly raising rubber stopper after cooling and adding an excess of the 0.01 *N* I. Then disconnect flask, wash tubes, rubber stopper, and sides of flask with the air-free wash soln; and titrate the excess of I with 0.01 *N*  $\text{Na}_2\text{S}_2\text{O}_3$ .

#### ZINC

##### *Gravimetric Method—Tentative*

(Minimum of 2 mg Zn)

#### 29.69

##### REAGENTS

(a) *Sodium or ammonium acetate soln.*—Dissolve 50 g of the salt in  $\text{H}_2\text{O}$  and make up to 100 ml.

(b) *Ferric chloride soln.*—Dissolve 10 g of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in 100 ml of  $\text{H}_2\text{O}$ .

#### 29.70

##### DETERMINATION

Boil the filtrate containing the Zn obtained after filtering off the  $\text{CuS}$ , 29.13, to expel  $\text{H}_2\text{S}$  and reduce volume to 250–300 ml, add a drop of methyl orange indicator and 5 g of  $\text{NH}_4\text{Cl}$ , and make alkaline with  $\text{NH}_4\text{OH}$ . Add  $\text{HCl}$  (1+9) dropwise to faintly acid reaction, then add 10–15 ml of the Na or  $\text{NH}_4$  acetate soln and pass in  $\text{H}_2\text{S}$  until precipitation is complete. Allow precipitate to settle, filter (clear filtrate is necessary), and wash precipitate twice with  $\text{H}_2\text{S}$  water. Dissolve precipitate on filter with a little  $\text{HCl}$  (1+3), wash filter with  $\text{H}_2\text{O}$ , boil combined filtrate and washings to expel  $\text{H}_2\text{S}$ , cool, and add distinct excess of Br water. Add 5 g of  $\text{NH}_4\text{Cl}$  and then  $\text{NH}_4\text{OH}$  until color of free Br disappears. Add  $\text{HCl}$  (1+3) dropwise until Br color just reappears; then add 10–15 ml of the Na or  $\text{NH}_4$  acetate soln and 0.5 ml of the  $\text{FeCl}_3$  soln, or enough to precipitate all the phosphates. Boil until all Fe is precipitated. Filter while hot and wash precipitate with  $\text{H}_2\text{O}$  containing a little Na acetate. Pass  $\text{H}_2\text{S}$  into the combined filtrate and washings until all the  $\text{ZnS}$ , which should be pure white, is precipitated. Filter thru weighed Gooch crucible, previously heated to constant weight, and wash with  $\text{H}_2\text{S}$  water containing a little  $\text{NH}_4\text{NO}_3$ . Dry crucible and its contents in oven, ignite at bright red heat ( $900^\circ$ ), cool, and weigh as  $\text{ZnO}$ . Calculate weight of metallic Zn, using factor 0.8034.

##### *Colorimetric Method (28)—Tentative*

#### 29.71

##### PRINCIPLES

This procedure involves the wet oxidation of the sample; elimination of Pb, Cu, Cd, Bi, Sb, Sn, Hg, and Ag as sulfides with added Cu as scavenger agent; simultaneous elimination of Co and Ni by extracting the metal complexes of  $\alpha$ -nitroso- $\beta$ -naphthol and dimethyl glyoxime, respectively, with  $\text{CHCl}_3$ ; extraction of the Zn dithizonate with  $\text{CCl}_4$ ; transfer of the Zn to dilute  $\text{HCl}$ ; and final extraction of the Zn dithizonate for color measurement.

#### 29.72

##### REAGENTS

(All  $\text{H}_2\text{O}$  must be redistilled from glass. Pyrex glassware should be used exclusively and must be scrupulously cleaned with hot  $\text{HNO}_3$ .)

(a) *Nitric acid.*—C.P., concentrated (redistilled if appreciably contaminated, altho not usually necessary).

(b) *Sulfuric acid*.—C.P., concentrated (should be tested if Zn contamination is suspected).

(c) *Ammonium hydroxide*.—C.P., concentrated (should be redistilled if appreciably contaminated).

(d) *Copper sulfate soln*.—Dissolve 8 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and dilute to 1 liter. 1 ml contains 2 mg of Cu.

(e) *Ammonium citrate soln*.—Dissolve 225 g of  $(\text{NH}_4)_2\text{HC}_6\text{H}_5\text{O}_7$  in  $\text{H}_2\text{O}$ , make alkaline to phenol red with  $\text{NH}_4\text{OH}$  (pH 7.4 first distinct color change), and add 75 ml in excess. Dilute to 2 liters. Extract this soln immediately before use as follows: Add slight excess of dithizone and extract with  $\text{CCl}_4$  until solvent layer is clear bright green. Remove excess dithizone by repeated extraction with  $\text{CHCl}_3$ , and finally extract once more with  $\text{CCl}_4$ . (It is essential that excess dithizone be entirely removed, otherwise Zn will be lost during elimination of Co and Ni.)

(f) *Dimethylglyoxime soln*.—Dissolve 2 g of the reagent in 10 ml of  $\text{NH}_4\text{OH}$  and 200–300 ml of  $\text{H}_2\text{O}$ , filter, and dilute to 1 liter.

(g)  *$\alpha$ -Nitroso- $\beta$ -naphthol soln*.—Dissolve 0.25 g in  $\text{CHCl}_3$  and dilute to 500 ml.

(h) *Chloroform*.—Redistilled.

(i) *Diphenylthiocarbazone (dithizone)*.—Dissolve 0.050 g in 2 ml of  $\text{NH}_4\text{OH}$  and 100 ml of  $\text{H}_2\text{O}$ , and extract repeatedly with  $\text{CCl}_4$  until solvent layer is clear, bright green color. Discard solvent layer and filter aqueous portion thru washed ashless paper. (This soln is best prepared as needed since it is only moderately stable, even when kept in dark and under refrigeration.)

(j) *Carbon tetrachloride*.—Redistilled.

(k) *Hydrochloric acid*.—0.04 N. Dilute required amount of concentrated C.P. acid with  $\text{H}_2\text{O}$  (redistilled acid may be used altho not usually required).

(l) *Standard zinc soln*.—Dissolve 0.500 g of pure granulated Zn in slight excess of dilute HCl and dilute to 1000 ml. For use dilute 10 ml of this stock soln to 1000 ml with 0.04 N HCl. 1 ml of this working standard contains 5 micrograms of Zn.

## 29.73

### PREPARATION OF SAMPLE

Weigh into Erlenmeyer flask of suitable size a representative sample not exceeding 25 g of material, estimated to contain 25–100 micrograms of Zn. If sample is a liquid, evaporate to small volume. Add  $\text{HNO}_3$  and heat cautiously until first vigorous reaction subsides somewhat, then add 2–5 ml of  $\text{H}_2\text{SO}_4$ . Continue heating, adding more  $\text{HNO}_3$  in small increments if necessary to prevent charring, until fumes of  $\text{SO}_3$  are evolved and soln remains clear and almost water white. Add 0.5 ml of  $\text{HClO}_4$  and continue heating until  $\text{HClO}_4$  has been almost completely removed. Allow to cool and dilute to ca 40 ml. (If necessary equipment is available, the wet digestion and subsequent sulfide separation may be advantageously carried out in small Kjeldahl flasks.)

## 29.74

### SEPARATION OF SULFIDE GROUP

To the  $\text{H}_2\text{SO}_4$  soln add 2 drops of methyl red indicator and 1 ml of the  $\text{CuSO}_4$  soln and neutralize with  $\text{NH}_4\text{OH}$ . Add sufficient HCl to make soln ca 0.15 N with respect to this acid (ca 0.5 ml excess in 50 ml of soln is satisfactory). The pH of the soln at this point as measured with glass electrode is 1.9–2.1. Pass stream of  $\text{H}_2\text{S}$  into soln until precipitation is complete. Filter thru fine textured paper (Whatman No. 42 or equivalent, which has been previously fitted to the funnel and washed with 5% HCl, then with redistilled  $\text{H}_2\text{O}$ ). Receive filtrate in 250 ml beaker and wash flask and filter with 3 or 4 small portions of  $\text{H}_2\text{O}$ . Boil filtrate gently until odor of  $\text{H}_2\text{S}$  can no longer be detected, then add 5 ml of saturated  $\text{Br-H}_2\text{O}$  and continue

boiling until Br has been expelled. Allow to cool, neutralize to phenol red with  $\text{NH}_4\text{OH}$ , and make slightly acid with HCl (excess of 0.2 ml of 1+1 HCl). Dilute resultant soln to volume. For optimum conditions of measurement, soln should contain 0.2–1.0 micrograms/ml.

**29.75****ELIMINATION OF NICKEL AND COBALT**

Transfer 20 ml aliquot of prepared soln to 125 ml separator; add 5 ml of the  $(\text{NH}_4)_2\text{HC}_6\text{H}_4\text{O}_7$ , buffer, 2 ml of the dimethylglyoxime soln, and 10 ml of the  $\alpha$ -nitroso- $\beta$ -naphthol soln; and shake for 2 min. Discard solvent layer and extract with 10 ml of  $\text{CHCl}_3$  to remove residual  $\alpha$ -nitroso- $\beta$ -naphthol. Discard solvent layer.

**29.76****ISOLATION AND ESTIMATION OF ZINC**

To aqueous phase following removal of Ni and Co, which at this point has pH of 8.0–8.2, add 2.0 ml of the dithizone soln and 10 ml of  $\text{CCl}_4$ , and shake 2 min. Allow phases to separate and remove aqueous layer as completely as possible, withdrawing liquid by means of pipet attached to vacuum line. Wash down sides of separator with ca 25 ml of  $\text{H}_2\text{O}$  and without shaking again draw off aqueous layer. Add 25 ml of the 0.04 N HCl and shake for 1 min. to transfer the Zn to acid-aqueous layer. Drain off and discard solvent, being careful to dislodge and remove drop that usually floats on surface. To acid soln add 5.0 ml of the  $(\text{NH}_4)_2\text{HC}_6\text{H}_4\text{O}_7$  soln and 10.0 ml of  $\text{CCl}_4$ . (The pH of soln at this point is 8.8–9.0.) Determine amount of dithizone to be added as follows: To separator containing 4.0 ml of the working Zn standard (20 micrograms) made up to 25 ml with the 0.04 N HCl, 5.0 ml of the citrate buffer, and 10.0 ml of  $\text{CCl}_4$ , add the dithizone reagent in 0.1 ml increments, shaking briefly after each addition until faint yellow color in aqueous phase indicates bare excess of the reagent. Multiply volume of dithizone soln required by 1.5 and add this volume (to nearest 0.05 ml) to all samples. Shake for 2 min. By means of pipet transfer exactly 5.0 ml of solvent layer to spectrophotometer cell, dilute with 10.0 ml of  $\text{CCl}_4$ , mix, and determine spectral transmission at 540 m $\mu$ . (Dilution may be made in clean, dry test tube if design of cell does not permit mixing directly.)

**29.77****PREPARATION OF STANDARD CURVES**

Prepare series of separators containing 0, 5, 10, 15, and 20 micrograms of Zn diluted to 25 ml with the 0.04 N HCl; add 5.0 ml of the citrate buffer, and proceed as with final extraction of Zn, 29.76.

Plot the transmittance on logarithmic scale against concentration and draw smooth curve thru the points. (Intercept of this curve may vary slightly from day to day, depending on actual concentration of dithizone employed in final extraction, but slope should remain essentially the same.)

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- (14) J. Assoc. Official Agr. Chem., **17**, 108 (1934); **18**, 315 (1935); **19**, 130 (1936).
- (15) Ibid., **21**, 695 (1938); **26**, 26 (1943).
- (16) Ibid., **20**, 191 (1937).
- (17) Ibid., **26**, 26 (1943).
- (18) Ibid., **20**, 622 (1937).
- (19) Ibid., **19**, 130 (1936); Ind. Eng. Chem., Anal. Ed., **11**, 400 (1939).
- (20) Ind. Eng. Chem., Anal. Ed., **12**, 218 (1940).
- (21) Ibid., **7**, 285 (1935).
- (22) Ibid., **14**, 904 (1942).
- (23) J. Assoc. Official Agr. Chem., **18**, 638 (1935); **22**, 341 (1939); **23**, 310 (1940).
- (24) Ibid., **22**, 346 (1939); **26**, 346 (1943).
- (25) Am. Chem. J., **18**, 705 (1896).
- (26) J. Assoc. Official Agr. Chem., **1**, 257 (1915).
- (27) Original communications, VIII Intern. Cong. Appl. Chem., **18**, 35 (1912).
- (28) J. Assoc. Official Agr. Chem., **27**, 325 (1944); **28**, 271 (1945).
- (29) Ibid., **28**, 269 (1945).

## 30. NUTS AND NUT PRODUCTS (1)

### 30.1

#### PREPARATION OF SAMPLE—TENTATIVE

Without delay, transfer all samples received in packages to glass-stoppered containers or Mason jars and keep in a cool, dark place. Prepare various samples for analysis as follows:

(a) *Fresh shelled nuts*.—Cut nuts into small pieces and weigh sample desired for analysis. Transfer weighed sample to mortar and grind to fine state of division with pestle. In transferring ground material from mortar to required flask, use a portion of the solvent to clean out mortar.

(b) *Shredded or prepared coconut*.—Transfer weighed sample to mortar and proceed as directed under (a).

(c) *Almond paste, kernel paste, peanut butter, etc.*—Transfer sample to Mason jar or beaker ca three times the size of sample and mix carefully with a stiff-bladed spatula or knife. *Almond paste and similar products containing added H<sub>2</sub>O must be freed of moisture before analysis.*

#### MOISTURE

### 30.2

*Method I.—Tentative.—See 27.3*

### 30.3

*Method II.—Tentative*

Weigh 2 g of the product into flat-bottomed dish. If necessary to secure a thin layer of the material, add a few ml of H<sub>2</sub>O, and mix thoroly. Dry at 70° under pressure not to exceed 100 mm of Hg until consecutive weighings made at intervals of 2 hours do not vary more than 3 mg.

### 30.4

**ASH—TENTATIVE.—See 34.9 or 34.10**

### 30.5

**CRUDE FIBER—TENTATIVE**

Use the fat-free material and proceed as directed under 27.30.

#### FAT, CONSTANTS OF FAT, AND PROTEIN

### 30.6

*Method I.—Tentative*

Weigh into 200 ml volumetric flask 2–3 g of material. Add 100 ml of CHCl<sub>3</sub> from pipet, washing down sample with stream of CHCl<sub>3</sub>. Stopper flask and shake frequently during 30 min. Filter soln thru 11 cm fluted paper, and as soon as 25 ml of soln has been filtered, pipet out two 10 ml portions, using same pipet. Transfer one aliquot to weighed crystallizing dish, 50×35 mm, and evaporate solvent on steam bath. Dry dish and contents at 100° for 30 min., cool, and weigh. Use weight obtained for calculating percentage of fat and I number of the fat. Determine refractive index of the dried residue. Transfer the other 10 ml portion to glass-stoppered flask or bottle, add 30 ml of Hanus soln, 31.18(a), and proceed as directed under 31.19. Complete filtration of the CHCl<sub>3</sub> extract. Transfer extracted residue to Kjeldahl flask, wash the volumetric flask thoroly with boiling H<sub>2</sub>O, and transfer washings to digestion flask. Determine N as directed under 2.26.  $N \times 6.25 = \text{protein}$ .

### 30.7

*Method II.—Tentative*

Proceed as directed under 19.23, using 300 ml Erlenmeyer receiving flask in place of the 150 ml flask. (A fritted Jena glass Büchner filter is more convenient



than the Knorr extraction tube.) To facilitate filtration, mix equal volume of Filter Cel with peanut butter. Determine constants of fat and protein as directed under 30.6.

#### SUGAR AND SALT

##### 30.8

##### *Method I.—Tentative*

Extract ca 10 g of sample in 8 oz. nursing bottle with two 100 ml portions of petroleum benzine, in each case shaking for 5 min., centrifuging, and pouring off supernatant liquid. Warm bottle to drive off remaining solvent and transfer dry residue to 100–150 ml separator. Complete transfer with mixture of 3 volumes of  $\text{CCl}_4$  and 1 volume of  $\text{CHCl}_3$ . Add more of the liquid and shake mixture vigorously. Wash down sides of separator, using total quantity of 60–80 ml of the liquid. Stopper separator and allow to stand overnight. Remove sugar and NaCl that have settled out by opening stopcock quickly, and if necessary pull out stopcock. Evaporate off liquid on steam bath or other warm place and dissolve residue in hot  $\text{H}_2\text{O}$ . Transfer soln to 100 ml volumetric flask with hot  $\text{H}_2\text{O}$ , cool, make to mark, and mix. Filter thru small, dry filter paper. Determine chlorides in 20 ml aliquot by titration with  $\text{AgNO}_3$ , using dichromate indicator. Determine reducing sugars before and after inversion as directed under 34.39.

##### 30.9

##### *Method II.—Tentative*

Weigh 10 g of the material on filter (with peanut butter thoroly mix 5 g of Filter Cel with weighed charge) and extract with suction 10 successive times at 3 min. intervals with 50 ml of petroleum benzine (b.p. below  $60^\circ$ ). (A fritted Jena glass Büchner filter is most convenient.) At beginning of each extraction stir soln well with glass rod flattened at end. After defatting, macerate well in porcelain mortar and transfer material with hot  $\text{H}_2\text{O}$  to 250 ml Pyrex or similar volumetric flask. If frothing occurs, add a few drops of alcohol, breaking up foam with glass rod. Pass hot  $\text{H}_2\text{O}$  thru filter and add to  $\text{H}_2\text{O}$  in flask until total volume is ca 200 ml. Digest in vessel of boiling  $\text{H}_2\text{O}$  for 15 min., cool under tap, and add ca 5 ml of a saturated neutral Pb acetate soln. Make to mark at room temp., shake well, transfer to centrifuge bottle, and whirl at 2000 r.p.m. for 15 min. Filter on  $18\frac{1}{2}$  cm folded filter, rejecting first 25 ml of filtrate. De-lead with K oxalate and again filter, rejecting first 25 ml of filtrate. Determine reducing sugars before and after inversion and calculate sucrose according to 34.30. Multiply results obtained by 0.97 to correct for volume of insoluble material. In the case of peanut butter, multiply by factor 0.95 to correct for volume occupied by Filter Cel and peanut butter. Determine chlorides as directed under 30.8.

##### 30.10

##### DEXTROSE OR D-GLUCOSE—TENTATIVE

Proceed as directed under 30.8 or 30.9 and calculate reducing sugars before inversion as dextrose.

#### PEANUT BUTTER

##### 30.11

##### PRELIMINARY PROCEDURE—TENTATIVE

Make microscopic examination to detect addition of starch or any off-grade material not identifiable chemically.

##### 30.12

##### STARCH—TENTATIVE

Weigh 4–5 g of sample by difference into 8 oz. nursing bottle and extract twice with 50 ml portions of petroleum benzine, in each case shaking for 5 min. Wash down

sides of bottle with petroleum benzine, centrifuge, and pour off solvent, disregarding opalescence. Warm bottle to drive off remaining solvent, and transfer residue to mortar and grind. Return the fine powder to bottle with aid of 100 ml of 10% NaCl soln. Shake bottle for 15 min., wash down sides with NaCl soln, centrifuge well, and pour off supernatant liquid, disregarding opalescence. Repeat this procedure twice. Extract once in same manner with 70% alcohol and then once with  $H_2O$ , shaking for 1–2 min. in each case. Drain bottle for several minutes, chill, and add from pipet 100 ml of HCl soln (20.5–21.0 g of HCl/100 ml) at temp. not higher than 15°. Shake vigorously for 3 min., centrifuge well, and pour off soln thru pledget of cotton in stem of funnel. Cool soln to temp. at which the HCl was added, and pipet off 50 ml into nursing bottle containing 115 ml of alcohol. Shake with whirling motion for 1 min., let stand for 2 min., centrifuge for 2 min., pour off thru a weighed Gooch crucible containing a thin pad of asbestos, and add 50 ml of 70% alcohol to precipitate. Stopper bottle, shake vigorously, wash down sides with the 70% alcohol, centrifuge lightly, and pour off thru crucible. Repeat once with 70% alcohol and once with alcohol. Dry crucible and contents for 1.5 hours at 130° in air, or for 5 hours at 98–100° in vacuo. Cover crucible, place in desiccator containing efficient desiccant, and weigh crucible as soon as it has attained room temp.

### ALMOND PASTE, KERNEL PASTE, ETC.

#### 30.13 SEPARATION AND PREPARATION OF THE OIL—TENTATIVE

Dry the paste in oven and extract repeatedly with petroleum benzine by rubbing in mortar and pouring off solvent thru filter. Evaporate benzine on steam bath and test extracted oil.

#### 30.14 *Bieber Test (2)*

Agitate 5 volumes of oil with 1 volume of mixture of equal parts, by weight, of  $H_2SO_4$ , fuming  $HNO_3$ , and  $H_2O$ . Pure almond oil does not change color; after standing for some time apricot kernel oil gives pink peach-blossom color, and peach kernel oil, faint pink coloration. It is advisable to prepare reagent fresh for each set of tests. It is doubtful whether less than 25% of apricot kernel oil can be detected.

#### 30.15 *Nitric Acid Test (3)*

On being shaken with  $HNO_3$ , almond oil remains colorless or becomes slightly yellow; apricot kernel oil assumes a color ranging from orange-yellow to red; and peach kernel oil becomes yellowish brown.

#### 30.16 *Kreis Test (4)*

Mix 1 volume of the oil in test tube with 1 volume of 0.10% soln of phloroglucinol in ether, and pour 1 volume of  $HNO_3$  down side of tube. Keep tube cold. A red ring forms at junction of two liquids when apricot kernel, sesame, or cottonseed oil is present. Almond oil gives no red color—or, at most, only a light pink.

The presence or absence of other oils (such as cottonseed, sesame, peanut, or olive) may be detected by the variation in constants and by characteristic tests. It is seldom that these oils are found unless added starch is present.

#### 30.17 MICROSCOPIC EXAMINATION

In connection with the microscopic examination of almond paste and other products containing ground almonds, attention is called to the following publications, which give detailed descriptions and illustrations of the tissue elements:

Young, W. J.—A Study of Nuts with Special Reference to Microscopic Identification, U. S. Dept. Agr. Bur. Chem. Bull. 160 (1912).

Hamig, E.—Z. Nahr. Genussm., 21, 577 (1911).

Pease, V. A.—Notes on the Histology of the Almond, J. Agr. Research, 41, 789–800 (1930).

Winton, Andrew L. and Kate B.—“The Structure and Composition of Foods,” Vol. 1, p. 476 (1932).

### SHREDDED COCONUT

#### 30.18

#### GLYCEROL—TENTATIVE

Extract with suction 4 times, 4 g of the shredded coconut (dried in vacuo at 70° for 5 or 6 hours) on filter (fritted Jena glass Büchner filter is most convenient), using for each extraction 50 ml of petroleum benzine (b.p. below 65°), and allowing 3 min. intervals between extractions. Use flattened glass rod for stirring. After removing fat, extract residue on filter with four 50 ml portions of absolute alcohol, allowing 3 min. intervals with stirring, as before. Make the absolute alcohol extract to 250 ml with absolute alcohol at room temp. Pipet 100 ml into 500 ml Erlenmeyer flask, and add 5 ml of H<sub>2</sub>O and a paste made by adding hot H<sub>2</sub>O to 2 or 3 g of Ba(OH)<sub>2</sub> in small mortar. Heat mixture on steam bath to boiling and boil ca 1 min.; transfer to 250 ml centrifuge bottle, and centrifuge at 2000 r.p.m. ca 5 min. Transfer the clear liquid to large porcelain dish and wash residue in centrifuge bottle with 50–75 ml of absolute alcohol, stirring with glass rod and centrifuging as before. Evaporate on steam bath at temp. below 70° to a few drops, or almost dryness. Transfer to 50 ml glass-stoppered cylinder with 10 ml of absolute alcohol and wash dish with two 5 ml portions of absolute alcohol. Further wash dish with three 10 ml portions of anhydrous ether, shaking glass-stoppered cylinder thoroly after each addition of the anhydrous ether. Transfer to sediment tube and centrifuge for 10 min. at a speed of 3200 r.p.m. Transfer clear soln in sediment tube to evaporating dish, preferably Pt, and wash sediment tube with 25 ml of mixture of absolute alcohol and anhydrous ether (2:3), stirring with glass stirring rod and centrifuging as before. Evaporate on steam bath at temp. of 85–90° to ca 5 ml, add 20 ml of H<sub>2</sub>O, and evaporate to ca 5 ml; repeat this operation twice. Transfer residue with hot H<sub>2</sub>O to 50 ml volumetric flask and proceed as directed under 33.76.

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- (2) Z. anal. Chem., 17, 264 (1878); Pharm. Centralb., 18, 315.
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- (4) Chem. Ztg., 26, 897 (1902).

## 31. OILS, FATS, AND WAXES

### 31.1

#### PREPARATION OF SAMPLE—OFFICIAL

Melt solid fats and filter by means of hot water funnel or similar apparatus. Make the different determinations on samples of this melted, homogeneous mass. Filter oils that are not clear. Keep oils and fats in cool place and protected from light and air, otherwise they will soon become rancid. Weigh out at one time as many portions as are needed for the various determinations, using small beaker or weighing buret.

#### MOISTURE AND VOLATILE MATTER (1)

### 31.2

#### *Vacuum Oven Method—Official*

Soften sample if necessary by means of gentle heat, taking care not to melt it. When sufficiently softened, mix thoroly with mechanical egg beater or other equally effective mechanical mixer.

Weigh 5 g ( $\pm 0.2$  g) of prepared sample into shallow glass moisture dish 6–7 cm in diam. and 4 cm deep. Dry to constant weight in vacuum oven (F.A.C. standard or equivalent) at uniform temp. not less than 20° nor more than 25° above boiling point of H<sub>2</sub>O at working pressure, which should not exceed 100 mm of Hg. Cool sample in efficient desiccator (30 min.) and weigh. Constant weight is attained when successive dryings for 1 hour periods show additional loss of not more than 0.05%. Report percentage loss in weight as moisture and volatile matter.

#### SPECIFIC GRAVITY (APPARENT)

#### *At 25/25°—Official*

### 31.3

#### STANDARDIZATION OF PYCNOMETER

Carefully clean pycnometer by filling it with saturated soln of CrO<sub>3</sub> in H<sub>2</sub>SO<sub>4</sub> and allowing to stand for several hours. Empty pycnometer and rinse thoroly with H<sub>2</sub>O; fill it with recently boiled H<sub>2</sub>O previously cooled to ca 20° and place in constant temp. bath at 25°. At end of 30 min. adjust level of the H<sub>2</sub>O to proper point on pycnometer and put the perforated cap or stopper in place; remove from bath, wipe dry with clean cloth or towel, allow to stand for 30 min., and weigh. Empty pycnometer, rinse several times with alcohol and then with ether, allow it to become perfectly dry, remove ether vapor, and weigh. Ascertain weight of contained H<sub>2</sub>O at 25° by subtracting weight of pycnometer from its weight when full.

### 31.4

#### DETERMINATION

Fill the clean, dry pycnometer with the oil previously cooled to ca 20°, place in constant temp. bath at 25° for 30 min., adjust level of oil to proper point on pycnometer, and put the cap or stopper in place; remove from bath, wipe dry, and weigh as directed under 31.3. Subtract weight of empty pycnometer from its weight when filled with oil and divide difference by weight of H<sub>2</sub>O at 25°, as determined under 31.3. The quotient is the sp. gr. at 25/25° (apparent).

### 31.5 TEMPERATURE CORRECTION FOR SPECIFIC GRAVITY OF OILS (s)—OFFICIAL

If the sp. gr. of the oil is determined at other than standard temp., the approximate sp. gr. at 25° may be calculated by means of following formula:

$G = G' + 0.00064(T - 25^\circ)$ , in which  $G$  = sp. gr. at  $25^\circ$ ;  $G'$  = sp. gr. at  $\frac{T}{25^\circ}$ ;  $T$  = temp. at which the sp. gr. was determined; and  $0.00064$  = mean correction for  $1^\circ$ .

*At Temperature of Boiling Water—Official*

## 31.6

## STANDARDIZATION OF FLASKS

(a) Weigh a 25–30 ml sp. gr. flask and fill with freshly boiled hot  $H_2O$ . Place in briskly boiling water bath for 30 min., replacing any evaporation from flask by addition of boiling  $H_2O$ . Insert stopper, previously heated to  $100^\circ$ , remove flask, cool, and weigh.

(b) The following formula may be used for calculating the weight of  $H_2O$  ( $W^T$ ) that a given flask will hold at  $T^\circ$  (weighed in air with brass weights at temp. of room) from weight of  $H_2O$  ( $W^t$ ) (weighed in air with brass weights at temp. of room) contained therein at  $t^\circ$ :

$$W^T = W^t \frac{d^T}{d^t} [1 + 0.000025(T - t)], \text{ in which}$$

$d^T$  = density of  $H_2O$  at  $T^\circ$ ; and  $d^t$  = density of  $H_2O$  at  $t^\circ$ .

## 31.7

## DETERMINATION

Fill the dry flask with the dry, hot, freshly filtered fat, which should be entirely free from air bubbles, and keep in water bath for 30 min. at temp. of boiling  $H_2O$ . Insert stopper, previously heated to  $100^\circ$ , cool, and weigh. Divide weight of contained fat by weight of contained  $H_2O$  previously found to obtain the sp. gr.

The weight of  $H_2O$  at boiling temp. must be determined under the barometric conditions prevailing at time the determination is made.

## INDEX OF REFRACTION

## 31.8

## GENERAL DIRECTIONS—OFFICIAL

Place instrument in such position that diffused daylight or some form of artificial light can readily be obtained for illumination. Circulate thru prisms a stream of  $H_2O$  of constant temp. Determine index of refraction with any standard instrument, reading oils at  $20$  or  $25^\circ$  and fats at  $40^\circ$ . The readings of the Zeiss butyro-refractometer on fats may be reduced to standard temp. by following formula (3):

$R = R' + 0.55(T' - T)$ , in which  $R$  = reading reduced to temp.  $T$ ;  $R'$  = reading at  $T'$ ;  $T'$  = temp. at which reading  $R'$  is made;  $T$  = standard temp.; and  $0.55$  = correction in scale divisions for  $1^\circ$ .

With oils the factor  $0.58$  is substituted in the formula for  $0.55$ , because they have a higher index of refraction. The readings of instruments that give the index of refraction directly can be reduced to standard temp. by substituting the factor  $0.00038$  for  $0.55$  in the formula. As the temp. rises the refractive index falls. The instrument used may be standardized with  $H_2O$  at  $20^\circ$ , the theoretical refractive index of  $H_2O$  at that temp. being  $1.3330$ . Any correction found should be made on all readings. The index of refraction varies with the density and in the same direction. If the results appear abnormal, compare the specific refractive power (4) with the normal.

Calculate the specific refractive power from the formula  $\frac{N-1}{D}$ , in which  $N$  equals

the refractive index and  $D$  the density. According to Proctor (5), the Lorenz formula,  $\frac{N^2 - 1}{(N^2 + 2)D}$ , gives much more satisfactory results than  $\frac{N - 1}{D}$ .

## 31.9

*I. By Means of Abbé Refractometer—Official*

To charge instrument, open double prism by means of screw head and place a few drops of sample on prism or, if preferred, open prisms slightly by turning screw head and pour a few drops of sample into funnel-shaped aperture between prisms. Close prisms firmly by tightening screw head. Allow instrument to stand for few minutes before reading is made, so that temp. of sample and instrument will be same.

Method of measurement is based upon observation of position of *border line of total reflection* in relation to the faces of a prism of flint glass. Bring this border line into field of vision of telescope by rotating the double prism by means of the alidade in following manner: Hold sector firmly and move alidade backward or forward until field of vision is divided into light and dark portion. Line dividing these portions is the "border line," and, as a rule, will not be a sharp line but a band of color. The colors are eliminated by rotating screw head of compensator until sharp, colorless line is obtained. Adjust border line so that it falls on point of intersection of cross hairs. Read refractive index of substance directly on scale of sector. Check correctness of instrument as directed under 31.8, or by means of quartz plate that accompanies it, using monobromonaphthalene, and make necessary correction in reading.

## 31.10

*Butyro-refractometer readings and indices of refraction*

READING	INDEX OF REFRACTION	READING	INDEX OF REFRACTION	READING	INDEX OF REFRACTION	READING	INDEX OF REFRACTION
40.0	1.4524	50.0	1.4593	60.0	1.4659	70.0	1.4723
40.5	1.4527	50.5	1.4596	60.5	1.4662	70.5	1.4726
41.0	1.4531	51.0	1.4600	61.0	1.4665	71.0	1.4729
41.5	1.4534	51.5	1.4603	61.5	1.4668	71.5	1.4732
42.0	1.4538	52.0	1.4607	62.0	1.4672	72.0	1.4735
42.5	1.4541	52.5	1.4610	62.5	1.4675	72.5	1.4738
43.0	1.4545	53.0	1.4613	63.0	1.4678	73.0	1.4741
43.5	1.4548	53.5	1.4616	63.5	1.4681	73.5	1.4744
44.0	1.4552	54.0	1.4619	64.0	1.4685	74.0	1.4747
44.5	1.4555	54.5	1.4623	64.5	1.4688	74.5	1.4750
45.0	1.4558	55.0	1.4626	65.0	1.4691	75.0	1.4753
45.5	1.4562	55.5	1.4629	65.5	1.4694	75.5	1.4756
46.0	1.4565	56.0	1.4633	66.0	1.4697	76.0	1.4759
46.5	1.4569	56.5	1.4636	66.5	1.4700	76.5	1.4762
47.0	1.4572	57.0	1.4639	67.0	1.4704	77.0	1.4765
47.5	1.4576	57.5	1.4642	67.5	1.4707	77.5	1.4768
48.0	1.4579	58.0	1.4646	68.0	1.4710	78.0	1.4771
48.5	1.4583	58.5	1.4649	68.5	1.4713	78.5	1.4774
49.0	1.4586	59.0	1.4652	69.0	1.4717	79.0	1.4777
49.5	1.4590	59.5	1.4656	69.5	1.4720	79.5	1.4780

## 31.11

*II. By Means of Zeiss Butyro-Refractometer—Official*

Place 2 or 3 drops of filtered sample on surface of lower prism. Close prisms and adjust mirror until it gives sharpest reading. If reading is indistinct after running  $H_2O$  of constant temp. thru instrument for some time, sample is unevenly distrib-

uted on surfaces of prism. As index of refraction is greatly affected by temp., use care to keep latter constant. Carefully adjust instrument by means of standard fluid that is supplied with it. Convert degrees of instrument into refractive indices from table, 31.10.

### MELTING POINT OF FATS AND FATTY ACIDS

*Wiley Method—Official*

#### 31.12

#### REAGENT

*Alcohol-water mixture.*—The sp. gr. should be the same as that of fat to be exam-

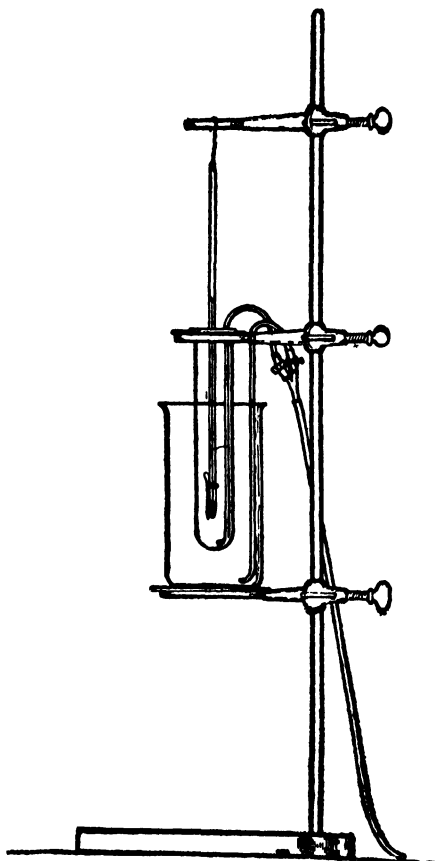


FIG. 48.—APPARATUS FOR DETERMINATION OF MELTING POINT

ined. Prepare by boiling, separately,  $H_2O$  and alcohol for 10 min. to remove gases that may be held in soln. While still hot pour the  $H_2O$  into test tube until it is almost half full. Nearly fill test tube with the hot alcohol, pouring it down side of

inclined tube to avoid too much mixing. If the alcohol is added after the  $\text{H}_2\text{O}$  has cooled, air bubbles will make mixture unfit for use.

## 31.13

## DETERMINATION

Allow the melted and filtered fat to fall a distance of 15–20 cm from dropping tube upon piece of ice or upon surface of cold Hg. The disks thus formed should be 1–1.5 cm in diam. and should weigh ca 200 mg. Remove disks when solid, and allow to stand 2–3 hours in order to obtain normal melting point.

Place a  $30 \times 3.5$  cm test tube, containing the alcohol-water mixture, in tall  $35 \times 10$  cm beaker containing ice and  $\text{H}_2\text{O}$ , and leave until mixture is cold. Drop disk of fat into tube. It will sink immediately to a point where density of the alcohol- $\text{H}_2\text{O}$  mixture is exactly equivalent to its own. Lower accurate thermometer, which can be read to  $0.1^\circ$ , into test tube until bulb is just above disk. In order to secure even temp. in all parts of the alcohol- $\text{H}_2\text{O}$  mixture around disk, stir gently with thermometer. Slowly heat the  $\text{H}_2\text{O}$  in beaker, constantly stirring it by means of air blast or other suitable device.

When temp. of the alcohol- $\text{H}_2\text{O}$  mixture rises to ca  $6^\circ$  below melting point of the fat, the disk of fat begins to shrivel and gradually rolls up into an irregular mass. Lower thermometer until fat particle is even with center of bulb. Rotate thermometer bulb gently and so regulate heat that ca 10 min. is required for last  $2^\circ$  increase in temp. As soon as fat mass becomes spherical, read thermometer. Remove tube from bath and again cool. Place in bath a second tube containing the alcohol- $\text{H}_2\text{O}$  mixture. The test tube is of sufficiently low temp. to cool bath to desired point. After first or preliminary determination, regulate temp. of bath so as to obtain a maximum of ca  $1.5^\circ$  above melting point of fat under examination.

If edge of disk touches sides of tube, make a new determination. Run triplicate determinations. The second and third results should agree closely.

## 31.14

*Capillary Tube Method (6)—Official*

Draw the melted fat or fatty acids into thin-walled capillary tube. Use column of fat 1–2 cm long, according to length of thermometer bulb. Seal one end of tube and cool on ice 12–15 hours. Attach capillary tube to bulb of accurate thermometer graduated to  $0.2^\circ$ , immerse in large test tube of  $\text{H}_2\text{O}$  surrounded by beaker of  $\text{H}_2\text{O}$ , and heat very slowly. An apparatus similar to that indicated in Fig. 48 may be used. Take as melting point the temp. at which the substance becomes transparent.

## TITER TEST (7)—OFFICIAL

## 31.15

## SPECIFICATIONS FOR TITER TEST THERMOMETERS

*Type*.—Etched stem, glass.

*Liquid*.—Mercury.

*Range and subdivision*.—Minus 2 to  $+68^\circ$  in  $0.2^\circ$ .

*Total length*.—385–390 mm.

*Stem*.—Constructed of suitable thermometer tubing of either plain or lens front type. Diameter, plain front type: 6–7 mm; diameter, lens front type: cross section of stem shall be such that it will pass thru 8 mm ring gage but will not enter a 5 mm slot gage.

*Bulb*.—Corning normal or equally suitable thermometric glass. Length, 15–25 mm; diameter, 5.5 mm to not greater than that of stem.

*Distance from bottom of bulb to  $-2^\circ$  mark*.—50–60 mm.

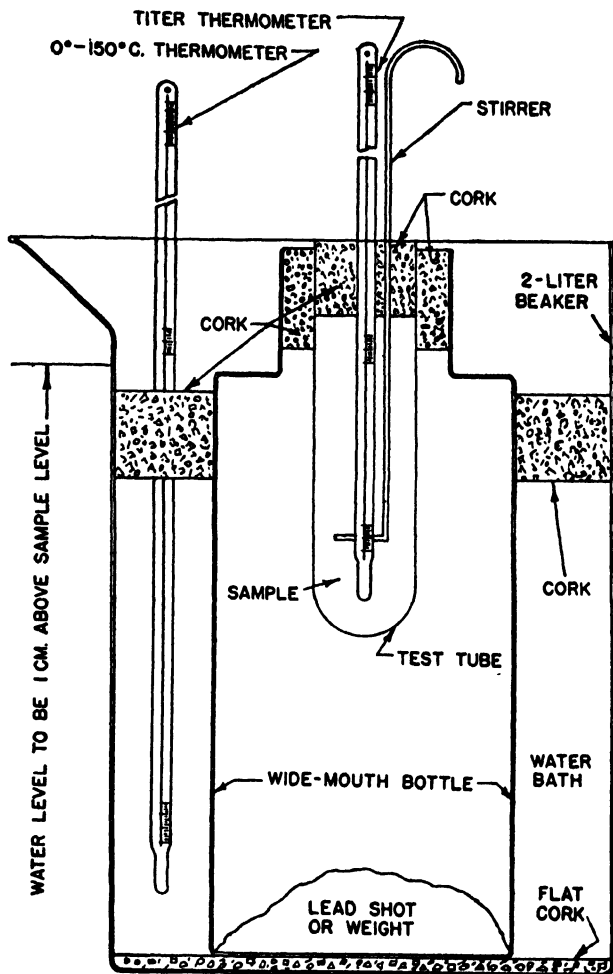
*Distance to  $68^\circ$  mark from top of thermometer*.—20–35 mm.



*Length of unchanged capillary.*—Between highest graduation and expansion chamber, 10 mm.

*Expansion chamber.*—To permit heating to at least 85°. Space above Hg to be evacuated or filled with N or other suitable gas.

*Top finish.*—Glass ring.



*Courtesy of Industrial Engineering Chemistry.*

FIG. 40—STIRRING TITER ASSEMBLY

*Graduation.*—All lines, figures, and letters to be clear-cut and distinct. Each degree mark to be longer than the remaining lines. Graduations to be numbered at zero and at each multiple of 2°.

*Immersion.*—45 mm.

**Marking.**—"A.O.A.C. Titer Test," a serial number, and the manufacturer's name or trademark shall be etched on stem. The words "45 mm immersion" shall also be etched on stem, and a line shall be etched around stem 45 mm above bottom of bulb.

**Scale error.**—The error at any point on scale shall not exceed  $0.2^{\circ}$ .

**Standardization.**—The thermometer shall be standardized at the ice point and at intervals of ca  $20^{\circ}$ , for the condition of 45 mm immersion, and for an average stem temp. of the emergent Hg column of  $25^{\circ}$ .

**Case.**—The thermometer shall be supplied in a suitable case on which shall appear the markings "A.O.A.C. Titer Test," " $-2^{\circ}$  to  $68^{\circ}$  in  $0.2^{\circ}$ ."

**NOTE:** For interpreting these specifications, the following definitions apply:

Total length is over-all length of finished instrument.

Diameter is that measured with ring gage or micrometer.

Length of bulb is distance from bottom of bulb to beginning of the enamel backing.

Top of thermometer is top of finished instrument.

### 31.16

#### APPARATUS

Stirring titer assembly, as shown in Fig. 49, consisting of a 2 liter beaker, a wide-mouth bottle (capacity 450 ml, height 190 mm, inside diameter of neck, 38 mm), titer test tube ( $25 \times 100$  mm) and a stirrer (2–3 mm outside diameter, one end bent in form of loop, 19 mm diameter).

### 31.17

#### DETERMINATION

Heat 110 g of glycerol-KOH soln (25 g of KOH in 125 g of C.P. glycerol) to  $150^{\circ}$  in 800 ml beaker and add 50 ml of the oil or melted fat, previously filtered if necessary to remove foreign substances. (Saponification often takes place almost immediately, but heating and frequent stirring should be continued for 15 min. Do not use temp. above  $150^{\circ}$ .) When saponification is complete, usually indicated by the perfectly homogeneous soln, cool slightly and add 200–300 ml of  $H_2O$ . After complete soln of the soap, add 50 ml of dilute  $H_2SO_4$  (16 ml of  $H_2SO_4$  in 70 ml of  $H_2O$ ), stirring during addition. Heat soln, with frequent stirring and addition of  $H_2O$  if necessary, until layer of fatty acids is completely melted and clear. Siphon off aqueous acid layer, add  $H_2O$  to the fatty acids, boil for 2 or 3 min., and again siphon off aqueous layer. Repeat treatment with  $H_2O$  until wash  $H_2O$  is neutral to methyl orange. Remove fatty acids so as not to include  $H_2O$  and filter while melted thru rapid filter paper. Heat to  $130^{\circ}$  on hot plate to remove traces of moisture and pour fatty acids into titer tube to height of 57 mm from bottom. If moisture is present in fatty acids, decant, refilter, and reheat.

Fill water bath and adjust temp. of  $H_2O$  to  $20^{\circ}$  for all samples with titers  $35^{\circ}$  or higher, and to  $15$ – $20^{\circ}$  below titer for samples with titers below  $35^{\circ}$ . ( $H_2O$  level should be 1 cm above sample level.) Place test tube containing fatty acids in assembly shown in Fig. 49. Insert thermometer to immersion mark and equidistant from sides of tube. Stir vertically with stirring rod at rate of 100 complete up and down motions per minute, starting agitation while temp. is at least  $10^{\circ}$  above titer point. (Stirrer should move thru vertical distance of ca 3.8 cm. If preferred, stirring may be performed by mechanical means.) Continue stirring until temp. remains constant for 30 seconds or begins to rise in less than a 30-second interval. Discontinue stirring immediately and observe rise in temp. Report as titer the highest point reached by thermometer. Duplicate determinations should normally agree within  $0.2^{\circ}$ .

## IODINE ABSORPTION NUMBER

(All reports should specify method used)

*Hanus Method—Official*

## 31.18

## REAGENTS

*Hanus iodine soln.*—Dissolve 13.2 g of pure I in 1 liter of acetic acid (99.5%) that shows no reduction with dichromate and  $\text{H}_2\text{SO}_4$ . Add enough Br to double the halogen content as determined by titration (ca 3 ml). The I may be dissolved by heating, but the soln should be cold when Br is added.

A convenient procedure for preparing the Hanus I soln is as follows: Measure 825 ml of acetic acid that has shown no reduction by dichromate test and dissolve in it with the aid of heat 13.615 g of I. Cool, and titrate 25 ml of this soln with 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3$ , 43.28. Measure another portion of 200 ml of the acetic acid and add 3 ml of Br. To 5 ml of this soln add 10 ml of the 15% KI soln, and titrate with the 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3$ . Calculate quantity of Br soln required to double halogen content of remaining 800 ml of I soln as follows:

$$A = \frac{B}{C}, \text{ in which}$$

$A$  = ml of Br soln required;  $B$  =  $800 \times$  thiosulfate equivalent of 1 ml of I soln; and  $C$  = thiosulfate equivalent of 1 ml of Br soln.

**EXAMPLE:** 136.15 g of I is dissolved in 8250 ml of acetic acid, and 30 ml of Br is dissolved in 2000 ml of acetic acid. Titrating 50 ml of the I soln against the standard  $\text{Na}_2\text{S}_2\text{O}_3$  shows that 1 ml of the I soln = 1.1 ml of the  $\text{Na}_2\text{S}_2\text{O}_3$  soln (0.0165 g of I). Titrating 5 ml of the Br soln shows that 1 ml of the Br soln = 4.6 ml of the  $\text{Na}_2\text{S}_2\text{O}_3$ . Then the quantity of Br soln required to double the halogen content of the re-

maining 8200 ml of I soln =  $\frac{8200 \times 1.1}{4.6}$ , or 1961 ml. Upon mixing the two solns

in this proportion, there is obtained a total volume of 10,161 ml, containing 135.3 g of I. In order to reduce this soln to the proper concn (13.2 g of I/liter), 10.161

$\times 13.2 = 134.1$ ;  $135.3 - 134.1 = 1.2$  g of I present in excess, or  $\frac{1.2 \times 1000}{13.2} = 91$  ml of

acetic acid, which must be added.

## 31.19

## DETERMINATION

Weigh ca 0.500 g of fat, or 0.250 g of oil (0.100–0.200 g in the case of oils that have high absorbent power), into 500 ml glass-stoppered flask or bottle. Dissolve the fat, or oil, in 10 ml of  $\text{CHCl}_3$ . With pipet add 25 ml of the Hanus I soln, allowing pipet to drain for definite time, and let stand for 30 min., shaking occasionally. (This time must be adhered to closely in order to obtain accurate results. The excess of I should be at least 60% of quantity added.) Add 10 ml of 15% KI soln, shake thoroly, and add 100 ml of freshly boiled and cooled  $\text{H}_2\text{O}$ , washing down any free I that may be found on stopper. Titrate the I with the 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3$ , adding it gradually, with constant shaking, until yellow color of the soln has almost disappeared. Add a few drops of starch indicator, 2.58(d), and continue titration until blue color has entirely disappeared. Toward end of titration, stopper bottle and shake violently, so that any I remaining in soln in the  $\text{CHCl}_3$  may be taken up

by the KI soln. Conduct two blank determinations along with that on sample. The number of ml of the 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3$  required by blank less quantity used in determination gives  $\text{Na}_2\text{S}_2\text{O}_3$  equivalent of the I absorbed by the fat or oil. Calculate percentage by weight of I absorbed and report as the I number (Hanus method).

#### *Wijs Method—Official*

##### 31.20

##### REAGENTS

*Wijs iodine soln.*—Dissolve 13 g of resublimed I in 1 liter of acetic acid (99.5%) and pass in washed and dried Cl gas until original  $\text{Na}_2\text{S}_2\text{O}_3$  titration of soln is not quite doubled. Use no more than slight excess of I and no excess of Cl. Preserve in glass-stoppered amber bottle sealed with paraffin until ready for use. Do not use Wijs soln that is more than 30 days old. Because of its unstable character  $\text{ICl}_3$  should not be used for preparation of the I soln (8).

##### 31.21

##### DETERMINATION

Weigh 0.10–0.50 g (depending on I number) of the melted and filtered sample into clean, dry, 16 oz., glass-stoppered bottle containing 15–20 ml of  $\text{CCl}_4$  or  $\text{CHCl}_3$ . With pipet add 25 ml of the I soln, allowing pipet to drain for definite time. The excess of I should be from 50 to 60% of quantity added, that is, from 100 to 150% of quantity absorbed. Moisten stopper with the 15% KI soln to prevent loss of I or Cl but guard against use of quantity sufficient to run down inside bottle. Let bottle stand in dark place for 30 min. at uniform temp. Add 20 ml of 15% KI soln and 100 ml of recently boiled and cooled  $\text{H}_2\text{O}$ . Titrate the I with 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3$ , 43.28, adding latter gradually and with constant shaking until yellow color of soln has almost disappeared. Add a few drops of starch indicator, 2.58(d), and continue titration until blue color has entirely disappeared. Toward end of reaction stopper bottle and shake violently so that any I remaining in soln in the  $\text{CCl}_4$  or  $\text{CHCl}_3$  may be taken up by the KI soln. Conduct two determinations on blanks, run in same manner as sample, but without any fat. Slight variations in temp. affect quite appreciably titer of the I soln as acetic acid has high coefficient of expansion. It is essential, therefore, that blanks and determinations on sample be made at same time. Number of ml of standard  $\text{Na}_2\text{S}_2\text{O}_3$  soln required by blank less quantity used in determination gives  $\text{Na}_2\text{S}_2\text{O}_3$  equivalent of the I absorbed by sample taken. Calculate percentage by weight of I absorbed and report as the I number (Wijs method).

#### THIOCYANOGEN NUMBER—TENTATIVE

##### 31.22

##### REAGENTS

(a) *Lead thiocyanate.*—Dissolve 331 g of finest C.P.  $\text{Pb}(\text{NO}_3)_2$  in 700 ml of  $\text{H}_2\text{O}$  and filter. Dissolve 194 g of C.P. KCNS in 500 ml of  $\text{H}_2\text{O}$  and filter. Slowly add the  $\text{Pb}(\text{NO}_3)_2$  soln to the KCNS soln with stirring, continue stirring for 30 min., and allow precipitate to settle. Decant supernatant liquid thru filter paper on Büchner funnel, using slight suction, and wash precipitate several times with  $\text{H}_2\text{O}$  by decantation. Transfer precipitate to Büchner funnel, using horn spoon and  $\text{H}_2\text{O}$ , and wash with  $\text{H}_2\text{O}$  until washings give no test for nitrates. Place precipitate on watch-glass and dry to constant weight (ca 7 days) in vacuum desiccator over  $\text{H}_2\text{SO}_4$ . The dried  $\text{Pb}(\text{SCN})_2$  should be white in color. Store in air-tight brown bottle and keep in the dark. Yield ca 260 g.

(b) *Thiocyanogen soln.*—0.2 *N*. Prepare anhydrous acetic acid by boiling gently in liter flask with ground-in glass air condenser, for ca 1 hour, 500 ml of acetic acid (at least 99.5%) with 40 ml of acetic anhydride. Attach a  $\text{CaCl}_2$  tube to end of condenser and allow the acid to cool to room temp.

*Solution 1.*—Weigh 8.4 g of dry Br into 250 ml graduated flask, dissolve in 100 ml of pure dry  $\text{CCl}_4$ , and fill flask to mark with anhydrous acetic acid.

*Solution 2.*—Pour 250 ml of anhydrous acetic acid on 25 g of the pure dry  $\text{Pb}(\text{SCN})_2$  in a colorless, dry, glass-stoppered liter bottle.

Add Soln 1 to Soln 2 in small quantities, giving Soln 2 a vigorous shaking after each addition and taking care that decoloration takes place before each addition of soln. After complete mixture of Solns 1 and 2 has been obtained, allow the suspension, consisting of precipitated  $\text{PbBr}_2$  and surplus  $\text{Pb}(\text{SCN})_2$ , to settle. Filter soln thru dry paper into a dry, brown, glass-stoppered bottle. Keep filtrate, which should be clear and colorless, or only slightly yellow, in the dark. If correctly prepared, 25 ml of this soln will require 48–52 ml of 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3$ , 43.28, for its iodometric titration. (The thiocyanogen soln will keep ca 1 week. After that time it begins to show a yellow color and a turbidity, and soon a fine yellow precipitate settles to bottom of bottle.)

## 31.23

## DETERMINATION

Weigh 0.1–0.2 g of the fat or oil (excess of the thiocyanogen reagent should be 150–200%) into 200 ml glass-stoppered bottle or flask. Add 25 ml of the thiocyanogen soln from pipet, rotate bottle gently until fat is dissolved, and allow to stand in dark 24 hours. Add 10 ml of 20% KI soln quickly and at one time, while shaking bottle to avoid hydrolysis of the thiocyanogen soln. Add 100 ml of  $\text{H}_2\text{O}$  and titrate the liberated I with the standardized 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3$  in usual manner, using starch indicator. Conduct at least two blank determinations along with the determination on sample. Subtract number of ml of  $\text{Na}_2\text{S}_2\text{O}_3$  soln required by sample from number required by blank. Multiply this difference by the I equivalent of the  $\text{Na}_2\text{S}_2\text{O}_3$  soln. Value obtained is quantity of I equivalent to the thiocyanogen absorbed by the fat or oil. Calculate percentage by weight and report as thiocyanogen number.

When iodine and thiocyanogen values are determined on the oil, calculate percentages of hypothetically pure triglycerides by following formulas:

*When no linolenin is present:*

$$Y = 1.246 \text{ I.V.} - 1.253 \text{ T.V.}; Z = 2.525 \text{ T.V.} - 1.348 \text{ I.V.}; \text{ and } S = 100 - (Y + Z).$$

*When linolenin is present:*

$$\begin{aligned} X &= 1.6610 \text{ T.V.} - 0.1332 \text{ I.V.} + 1.3056 S - 130.56; \\ Y &= 1.4137 \text{ I.V.} - 3.3449 \text{ T.V.} - 1.6441 S + 164.41; \text{ and } \\ Z &= 1.6839 \text{ T.V.} - 1.2805 \text{ I.V.} - 0.6615 S + 66.15; \text{ where } \\ X &= \% \text{ linolenic acid glycerides; } Y = \% \text{ linoleic acid glycerides; } \\ Z &= \% \text{ oleic acid glycerides; } S = \% \text{ saturated acid glycerides and unsaponifiable matter; } \text{I.V.} = \text{I number and T.V.} = \text{thiocyanogen number.} \end{aligned}$$

When the iodine and thiocyanogen values are determined on the fatty acids, calculate as follows:

*When no linolenic acid is present:*

$S = 100 - (Y + Z)$ ;  $Y = 1.194 \text{ I.V.} - 1.202 \text{ T.V.}$ ; and  
 $Z = 2.421 \text{ T.V.} - 1.293 \text{ I.V.}$

*When linolenic acid is present:*

$X = 1.5902 \text{ T.V.} - 0.1290 \text{ I.V.} + 1.3040 S - 130.40$ ;  
 $Y = 1.3565 \text{ I.V.} - 3.2048 \text{ T.V.} - 1.6423 S + 164.23$ ; and  
 $Z = 1.6146 \text{ T.V.} - 1.2275 \text{ I.V.} - 0.6617 S + 66.17$ ; where  
 $X = \%$  linolenic acid;  $Y = \%$  linoleic acid;  $Z = \%$  oleic acid;  
 $S = \%$  saturated acid; I.V. = I number and T.V. = thiocyanogen number.

#### SAPONIFICATION NUMBER (KOETTSTORFER NUMBER)—OFFICIAL

##### 31.24

##### REAGENT

*Alcoholic potassium hydroxide soln (9).*—(1) Reflux 1.2 liters of alcohol for 30 min. in distilling flask with 10 g of KOH and 6 g of granulated Al (or foil). Distil, and collect 1 liter after discarding first 50 ml. Dissolve 40 g of high-grade KOH in this liter of alcohol. Keep soln in glass-stoppered bottle. Or (2) crush 40 g of high-grade KOH in 7 or 8" mortar. Add 45 g of granulated CaO and grind mixture to powder. From liter of alcohol add 100 ml to mortar and transfer to flask, rinsing mortar with several more portions. Add remainder of alcohol to flask, shake mixture at least 5 min., and invert a beaker over neck of flask. Repeat shaking several times during day. Next morning filter soln into clean, dry, glass-stoppered bottle.

##### 31.25

##### DETERMINATION

Weigh accurately ca 5 g of filtered sample into 250–300 ml Erlenmeyer flask. Pipet 50 ml of the alcoholic KOH soln into flask, allowing pipet to drain for definite time. Connect flask with air condenser and boil until fat is completely saponified (ca 30 min.). Cool, and titrate with 0.5 *N* HCl, 43.7–43.8, using phenolphthalein indicator. Conduct blank determination along with that on sample, using same pipet for measuring the KOH soln and draining for same length of time. Subtract number of ml of 0.5 *N* HCl obtained in determination on sample from number obtained on blank to obtain the ml of 0.5 *N* HCl equivalent to the KOH used in saponification of sample taken. Calculate and report as saponification number (mg of KOH required to saponify 1 g of fat).

##### 31.26

##### SOLUBLE ACIDS—OFFICIAL

Place flask used under 31.25 and its contents on water bath and evaporate the alcohol. Add that quantity of 0.5 *N* HCl which is equivalent to quantity of KOH used for saponification of the sample under 31.25 and 1 ml more (quantity of 0.5 *N* HCl to be added = titration for blank—titration for sample + 1 ml), and place flask on steam bath until separated fatty acids form clear layer on upper surface of liquid. Fill flask to neck with hot H<sub>2</sub>O and cool contents in ice H<sub>2</sub>O until cake of fatty acids is thoroly hardened. Pour liquid contents of flask thru filter into liter flask, refill flask with hot H<sub>2</sub>O, and set on steam bath until fatty acids collect at surface. Cool by immersing in ice H<sub>2</sub>O and again filter liquid into the liter flask. Repeat this treatment with hot H<sub>2</sub>O 3 times, cooling and collecting washings in the liter flask after each treatment. Titrate combined washings with 0.1 *N* NaOH, 43.4–43.5, using phenolphthalein indicator. Subtract 5 (corresponding to excess of 1 ml of 0.5 *N* acid)

from number of ml of 0.1 *N* NaOH used and multiply by 0.0088 to obtain weight of soluble acids as butyric acid. Calculate percentage of soluble acids.

## 31.27

## INSOLUBLE ACIDS (HEHNER NUMBER)—OFFICIAL

Allow flask containing the cake of insoluble fatty acids from 31.26 and the paper thru which the soluble fatty acids have been filtered to drain and dry for 12 hours. Transfer cake, together with as much of fatty acids as can be removed from filter paper, to weighed, wide-mouth beaker flask. Place funnel containing filter in neck of flask and wash paper thoroly with hot absolute alcohol. Remove funnel, evaporate alcohol, dry for 2 hours at 100°, cool in desiccator, and weigh. Again dry for 2 hours, cool, and weigh. If there is any considerable decrease in weight, re-heat for 2 hours, cool, and weigh again. Calculate percentage of insoluble fatty acids.

SOLUBLE AND INSOLUBLE VOLATILE ACIDS (REICHERT-MEISSL AND POLENSKE VALUES) (10)—OFFICIAL

## 31.28

## REAGENTS

(a) *Sodium hydroxide soln.*—(1+1). Protect soln from contact with CO<sub>2</sub>. Allow soln to settle and use only clear liquid.

(b) *Pumice stone.*—Heat small pieces to white heat, plunge into H<sub>2</sub>O, and keep there until used.

(c) *Glycerol-soda soln.*—Add 20 ml of the 1+1 NaOH soln to 180 ml of pure concentrated glycerol.

## 31.29

## DETERMINATION

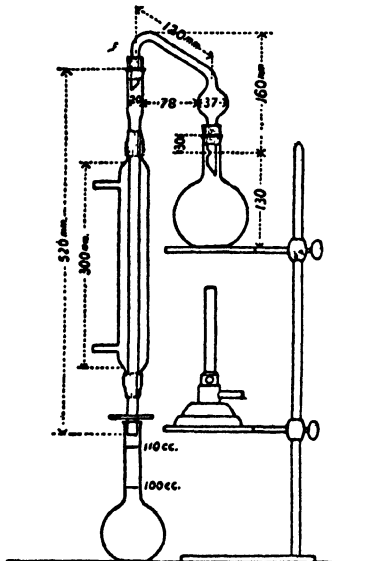


FIG. 50.—APPARATUS FOR DETERMINATION OF POLENSKE NUMBER

Weigh accurately 5 g of sample to be tested into clean, dry, 300 ml flask; add 20 ml of the glycerol-soda soln and heat over flame or asbestos plate until complete saponification occurs, as shown by mixture becoming perfectly clear. If foaming occurs, shake flask gently. Add 135 ml of recently boiled H<sub>2</sub>O, dropwise at first to prevent foaming, then add 6 ml of H<sub>2</sub>SO<sub>4</sub> (1+4) and a few fragments of pumice stone. Distil without previously melting the fatty acids, using apparatus of approximate dimensions illustrated in Fig. 50. Rest flask on piece of asbestos board having a hole 5 cm in diameter in center, and so regulate flame as to collect 110 ml of distillate in as near 30 min. as possible and to allow distillate to drip into receiving flask at temp. not higher than 18–20°.

When distillation is complete, substitute for receiving flask a 25 ml cylinder to collect any drops that may fall after flame has been removed. After mixing without violent shaking, immerse flask containing distillate almost completely in H<sub>2</sub>O at 15° for 15 min., filter the 110 ml of distillate thru dry filter

paper 9 cm in diam., and titrate 100 ml of filtrate with 0.1 *N* NaOH soln, 43.4–43.5, using phenolphthalein (1% alcoholic soln) as indicator. The pink color should

remain unchanged for 2 or 3 min. The Reichert-Meissl value is the number of ml of 0.1 *N* NaOH soln used times 1.1 after this result is corrected for figure obtained in blank determination.

Remove remainder of soluble acids from insoluble acids upon filter paper by washing with 3 successive 15 ml portions of H<sub>2</sub>O, previously passed thru the condenser, the 25 ml cylinder, and the 110 ml receiving flask. Dissolve the insoluble acids by passing 3 successive 15 ml portions of neutral alcohol, 95% by volume, thru filter paper, each portion having previously passed thru the condenser, the 25 ml cylinder, and the 110 ml receiving flask. Titrate combined alcoholic washings with 0.1 *N* NaOH soln, using the phenolphthalein as indicator. Polenske value equals number of ml of 0.1 *N* NaOH soln required for titration, after this result is corrected for titration obtained in blank determination.

NOTE.—Unless these directions are followed in every detail as described, satisfactory results cannot be obtained.

## 31.30

## KIRSCHNER VALUE (11)—OFFICIAL

To 100 ml of the Reichert-Meissl distillate, 31.29, in 200 ml Erlenmeyer flask, add 6 drops of phenolphthalein soln and titrate to very faint pink with a 0.1 *N* Ba(OH)<sub>2</sub> soln. Add 0.3 g of finely powdered Ag<sub>2</sub>SO<sub>4</sub>. During next hour shake mixture frequently, filter, and transfer 100 ml of filtrate to 300 ml flask. Add 10 ml of H<sub>2</sub>SO<sub>4</sub> (1+40), 35 ml of H<sub>2</sub>O, and a piece of Al wire or several small pieces of pumice stone, 31.28(b). Distil 110 ml in ca 20 min., using Polenske apparatus, Fig. 50. Titrate 100 ml of distillate with 0.1 *N* Ba(OH)<sub>2</sub> soln, make blank determination, and after correcting number of ml of alkali used, calculate the Kirschner value according to following

formula:  $K = \frac{A \times 121 (100 + B)}{10,000}$ , in which *A* = corrected Kirschner titration and

*B* = number of ml of standard alkali soln to neutralize the 100 ml Reichert-Meissl distillate.

Butter fat gives Kirschner values from 19 to 26, coconut oil gives an average of 1.9, and palm kernel oil 1.0, whereas the majority of other fats and oils give values from 0.1 to 0.2.

## SATURATED AND UNSATURATED FATTY ACIDS

## 31.31

## Lead Salt-Ether Method (12)—Official

(Not applicable to fats and oils that contain erucic, elaeostearic, chaulmoogric, hydnocarpic or similar acids; to hydrogenated products that contain notable quantities of iso-oleic acid; nor to coconut or palm kernel oils that contain notable quantities of the lower fatty acids that give ether-soluble Pb salts.)

Weigh accurately 10 or 20 g of sample into 200 ml Erlenmeyer flask. Add 30 ml of alcohol and 8 ml of KOH soln (1+1). Mix thoroly and heat on steam bath for ca 30 min. Add slight excess of acetic acid (1+2), using phenolphthalein as indicator, and then add sufficient quantity of 15% KOH soln while rotating flask to produce distinct pink color. Heat to boiling in liter flask 60 ml (120 ml for 20 g of sample) of 20% Pb acetate soln and same quantity of H<sub>2</sub>O. Add the neutralized soap soln cautiously to avoid any loss, rinsing saponification flask with 5 ml of alcohol, then with small volumes of hot H<sub>2</sub>O. Boil mixture gently ca 5 min., shake thoroly, and cool under running H<sub>2</sub>O, rotating flask to cause all precipitated Pb soaps to adhere to sides and bottom of flask. When mixture is cold pour off aqueous soln into large beaker in order to examine soln for particles of Pb soap. (Usually soln is slightly



turbid owing to some basic Pb acetate, and no particles or globules of Pb soap are seen.) Wash flask and Pb soap twice with cold  $\text{H}_2\text{O}$  and allow flask to drain for 10 min. Remove last drops of  $\text{H}_2\text{O}$  by means of thin roll of filter paper held by forceps, being careful to press paper only lightly against precipitate. Add ca 120 ml of ether and shake by rotating flask ca 5 min.

Connect flask with reflux condenser and boil contents gently until the Pb soap is completely disintegrated or dissolved. Remove flask and rinse down sides with sufficient ether to make final volume ca 150 ml. Invert close fitting beaker over neck of flask and place in refrigerator for at least 15 hours. Place 7 cm ordinary filter paper in Büchner funnel of 7.5 cm diameter, turn on full suction, and fit hardened filter paper cut to 8 cm in diameter as snugly as possible to sides of funnel. Decant ether soln from separated Pb soaps, using only sufficient suction to draw ether thru filter. (Too much suction causes ether to evaporate so rapidly that filter may become clogged with separated unsaturated acids, Pb soaps, or ice.)

Transfer precipitate to filter by rinsing flask with small portions of ether. During filtration keep funnel covered as much of the time as possible to prevent evaporation of ether. If at any time filtration proceeds so fast as to cause the mass of Pb soap to crack, close cracks by pressing with small spoon or spatula; otherwise precipitate cannot be properly washed. Rinse spoon free from precipitate and wash precipitate 8 or 10 times with ether, finally allowing suction to continue until precipitate cracks into numerous pieces. Without delay, separate with spoon as much of precipitate as possible and transfer it without loss to 500 ml separator containing ca 50 ml of ether, washing off any precipitate adhering to spoon and neck of separator with ether. Transfer filter paper to liter flask. Shake contents of separator thoroly to disintegrate lumps of Pb salt and allow to stand ca 20 min. Add 20 ml of HCl previously diluted with 10 ml of  $\text{H}_2\text{O}$  and shake thoroly for 2 min. to decompose all the Pb soap. Add 5–10 ml of HCl (2+1) to the liter flask containing filter paper, shake thoroly to decompose any precipitate adhering to flask and filter; then wash into the separator with small alternate portions of ether and  $\text{H}_2\text{O}$  until all fatty acids and  $\text{PbCl}_2$  are removed from flask. Again shake contents of separator with rotary motion and allow to stand for 10 min. Withdraw lower aqueous soln slowly, taking precautions not to remove any emulsion or undecomposed Pb soap. When Pb soap is present (shown in form of lumps that float on top of aqueous soln), add 10 ml of HCl and shake again; add ca 20 ml of  $\text{H}_2\text{O}$ , shake, and allow mixture to stand until layers have separated. Withdraw aqueous soln and wash ether with successive 25 ml portions of  $\text{H}_2\text{O}$  until washings are free from HCl. Dehydrate ether with ca 2 g of anhydrous  $\text{Na}_2\text{SO}_4$  and transfer soln to weighed 300 ml Erlenmeyer flask. Rinse separator and  $\text{Na}_2\text{SO}_4$  with several small portions of ether to remove all fatty acids, taking care not to allow any of the  $\text{Na}_2\text{SO}_4$  to fall into weighed flask. Distil ether, avoiding any loss of the fatty acids, and heat in oven at ca  $110^\circ$  until weight is constant. Obtain weight of saturated acids and save them for later investigation.

Transfer the ether soln of the soluble Pb soaps to 500 ml or 1000 ml separator, rinsing the Büchner funnel and filter flask with small quantities of ether. Add a mixture of 30 ml of HCl and 75 ml of  $\text{H}_2\text{O}$  and shake with rotary motion for 2 min. After allowing mixture to stand for 10 min., slowly withdraw the aqueous soln into beaker. If drops of the ether soln are entrapped by the  $\text{PbCl}_2$  precipitate and are removed with it, decant soln from the precipitated  $\text{PbCl}_2$  that has settled into separator. Rinse beaker and precipitate with small quantities of ether, adding washings to separator. Rotate contents of separator and allow to stand for 10 min. Withdraw the aqueous soln and wash ether with successive 50 ml portions

of  $H_2O$  until the  $HCl$  is removed. Transfer ether soln to 300 ml weighed Erlenmeyer flask. Distil the ether and place flask in oven heated to ca  $110^\circ$  ca 1 hour, while passing stream of  $CO_2$  into flask to prevent oxidation of unsaturated acids. Cool in atmosphere of  $CO_2$ . When cold, remove the  $CO_2$  and weigh. Repeat this treatment until constant weight is obtained.

Determine in duplicate the I numbers of both saturated and unsaturated acid fractions. (The I number of the saturated acid fraction is due to presence of some unsaturated acid.)

To correct for unsaturated acids present in fraction of saturated acids use following formula:

$$\frac{\text{I No. of saturated acid fraction}}{\text{I No. of unsaturated acid fraction}} \times 100 = A \quad (\text{percentage of unsaturated acids in saturated acid fraction}).$$

Obtain correct value by means of formula  $\frac{A \times B}{100}$ , in which  $B$  is percentage of

the impure saturated acids (as found by analysis). Subtract this correction from percentage of impure saturated acids and add it to percentage of unsaturated acids actually determined.

#### FREE FATTY ACIDS IN CRUDE AND REFINED OILS

##### 31.32

##### *N. C. P. A. Methods—Official*

(a) *In crude oils.*—Weigh 7.05 g of well-mixed oil into 250 ml flask or 4 oz. bottle. Add 2 ml of 1% alcoholic phenolphthalein soln to 50 ml of denatured alcohol, SD Formula 30 (1 vol. of methanol and 10 vols. of ethyl alcohol), or isopropyl alcohol, and add sufficient 0.1  $N$   $NaOH$ , 43.4–43.5, to give faint pink color. Add this mixture to the oil in the flask or bottle. Titrate with 0.25  $N$   $NaOH$  soln with vigorous shaking until a permanent faint pink color appears and persists for at least 1 min. Report as percentage of free fatty acids expressed as oleic acid. The number of ml of the 0.25  $N$   $NaOH$  used in the titration corresponds to this percentage.

(b) *In refined oils.*—Put ca 50 ml of alcohol (Formula 30) into clean, dry 150 ml flask and add a few drops of refined oil and 2 ml of 1% phenolphthalein soln. Place flask in  $H_2O$  at  $60$ – $65^\circ$  until warm, and add a sufficient quantity of the 0.1  $N$   $NaOH$  soln to produce a faint permanent pink color. Weigh 56.4 g of the refined oil into the neutralized alcohol and titrate, occasionally warming and violently shaking mixture until the same faint permanent pink color appears in the supernatant alcohol. Multiply number of ml of 0.1  $N$   $NaOH$  by 0.05 and report as percentage of free fatty acids expressed as oleic acid.

#### ACETYL VALUE (15)—OFFICIAL

##### 31.33

##### *Acetylation*

Boil 50 ml of sample with 50 ml of freshly distilled acetic anhydride under reflux condenser for 2 hours. Pour mixture into 500 ml of  $H_2O$  in beaker and boil for 15 min., while bubbling a stream of air or of  $CO_2$  thru the soln to prevent bumping. Siphon off the  $H_2O$ , add 500 ml more of  $H_2O$ , and boil again for 15 min. Repeat siphoning and boil for 15 min. with a third 500 ml portion of  $H_2O$ . Allow mixture to cool and separate aqueous layer, which should be neutral to litmus. Transfer the acetylated oil to separator and wash with two 200 ml portions of warm  $H_2O$ . Separate as much of the  $H_2O$  as possible, add 5 g of anhydrous  $Na_2SO_4$  to the acetylated

oil, and let stand for 1 hour, agitating occasionally to assist the drying. Filter thru dry folded filter, preferably in oven heated to 100–110°, and keep filtered oil in oven until it is completely dry. The acetylated product should be a clear, brilliant oil.

### 31.34

#### *Saponification*

Weigh accurately 2–2.5 g each of the acetylated oil and of the untreated oil into separate 250 ml Erlenmeyer flasks. Add to each flask exactly 25 ml of alcoholic KOH soln, 31.24, and reflux for 1 hour. Titrate the warm solns with 0.5 N HCl, using phenolphthalein as indicator. Titrate in same way two 25 ml portions of the alcoholic KOH soln. From the mean of these two results, which should be in very close agreement, deduct the volume of the standard HCl required for titration of the acetylated and of the untreated oil, and from results so obtained calculate the saponification number (mg of KOH required to saponify 1 g of product) of each. Calculate the acetyl value by means of the following formula:

$$A = \frac{S' - S}{1 - 0.00075S}, \quad \text{in which}$$

$A$  = acetyl value;  $S$  = saponification number of oil; and  $S'$  = saponification number of the acetylated oil.

## CHOLESTEROL AND PHYTOSTEROL IN MIXTURES OF ANIMAL AND VEGETABLE FATS

### 31.35

#### *Alcohol Extraction Method (14)—Tentative*

(Not applicable in presence of hydrogenated soybean oil)

Introduce 200–300 g of the melted fat into flat-bottomed liter flask. Close neck of flask with 3-holed stopper and insert thru these holes: (1) A reflux condenser; (2) a right-angled glass tube, one arm of which reaches to a point 6 mm above surface of melted fat, the other being closed a short distance from flask by means of short piece of rubber tubing and pinch-cock; (3) a glass tube bent so that one arm reaches to bottom of flask and the other serves as delivery tube for a 700 ml round-bottomed flask containing 500 ml of alcohol.

Place flasks containing melted fat and alcohol on steam bath and heat so that alcohol vapor passes thru the melted fat in the liter flask and is condensed in the reflux condenser, finally collecting in layer over the melted fat. After all alcohol has passed in this manner into flask containing the fat, disconnect flask from which the alcohol has been distilled and attach tube to the short piece of rubber tubing attached to right-angled glass tube, see (2) above, and siphon alcohol layer back into alcohol distillation flask. Reconnect as at first and again distil alcohol as in first operation. When all alcohol has been distilled, siphon it again into distillation flask and extract in same manner a third time.

Discard fat and retain alcohol, which now contains practically all cholesterol and phytosterol originally present in fat. Concentrate alcoholic soln to ca 250 ml, and to boiling liquid add 20 ml of KOH soln (1+1). Boil for 10 min. to insure complete saponification of fat, cool to room temp., and pour into large separator containing 500 ml of warm ether. Shake to insure thoro mixing and add 500 ml of H<sub>2</sub>O. Rotate separator gently to avoid formation of extremely stubborn emulsions, but mix the H<sub>2</sub>O thoroly with the alcohol-ether-soap soln. A clear, sharp separation

takes place at once. Draw off soap soln and wash ether layer with 300 ml of  $H_2O$ , avoiding shaking. Repeat washing of ether soln with small quantities of  $H_2O$  until all soap is removed. Transfer ether layer to flask and distil ether until volume of liquid remaining in flask measures ca 25 ml. Transfer this residue to tall 50 ml beaker and continue evaporation until all ether is driven off and residue is perfectly dry. If desired, weighed beaker may be used and weight of unsaponifiable matter determined at this point.

Add 3–5 ml of acetic anhydride to residue in beaker, cover beaker with watch-glass, and heat to boiling over free flame. After boiling for a few seconds, remove beaker from flame, cool, and add 35 ml of alcohol, 60% by volume. Mix contents of beaker thoroly, filter off alcoholic soln, and wash precipitate with the 60% alcohol. Dissolve precipitate on filter with stream of hot alcohol, 80% by volume, and wash insoluble portion well with the 80% alcohol. Acetates of cholesterol and phytosterol are dissolved, while the greater portion of impurities present (including paraffin and paraffin oil) remains on filter. Cool combined filtrate and washings to temp. of 10–12° and allow to stand at that temp. for 2–3 hours. During this time the acetates of cholesterol and phytosterol crystallize from the soln. Collect the crystals upon filter, wash with cold alcohol, 80% by volume, and then dissolve in minimum quantity of hot absolute alcohol. Collect the alcoholic soln of the acetates in small glass evaporating dish, add 2 or 3 drops of  $H_2O$  to soln, and heat if not perfectly clear. Allow alcohol to evaporate spontaneously, stirring contents of dish occasionally to mix deposit of crystals that form upon edges with main body of liquid. As soon as a good deposit of crystals has formed, collect them upon hardened filter; wash twice with cold alcohol, 90% by volume; and dry by suction, drying finally at 100° for 30 min. Determine melting point in apparatus shown in Fig. 48, using  $H_2SO_4$  in outer beaker and glycerol in inner tube.

The melting point of first crop of crystals usually gives definite information as to presence or absence of phytosterol, but the conclusion indicated should be confirmed by recrystallizing the crystals from absolute alcohol and again determining melting point. If crystals are pure cholesteryl acetate, the melting point of second crop should agree closely with that of first. If phytosteryl acetate is present, however, a higher melting point will be noted, as phytosteryl acetate is less soluble in alcohol than cholesteryl acetate. The melting point of cholesteryl acetate is 114°; that of phytosteryl acetate, 125–137°.

### 31.36

#### *Digitonin Method (15)—Tentative*

Shake vigorously 50 g of the oil, or fat, for 15 min. in separator with 20 ml of a 1% soln of digitonin in alcohol. Allow mixture to stand for a time until emulsion separates. Lower or fat layer should be quite clear while alcohol layer contains a bulky, flocculent precipitate. Draw off as much of fat as possible, avoiding any loss of precipitate. Add 100 ml of ether to alcohol layer and filter mixture. After drying in air wash precipitate with ether until free from fat, transfer to tall 50 ml beaker, and add 2–3 ml of acetic anhydride. Cover beaker with watch-glass. Boil slowly over low flame for 30 min. After cooling, add 30–35 ml of alcohol, 60% by volume, and mix contents of beaker thoroly. Filter the alcohol soln. Wash precipitate with the 60% alcohol, then dissolve on filter with stream of hot alcohol, 80% by volume, from wash bottle, and set filtrate aside in cool place (10° or below). After acetates have crystallized out of this soln filter them off, recrystallize from absolute alcohol, dry, and determine melting point of each crop of crystals as directed under 31.35.

## UNSAAPONIFIABLE RESIDUE

## F. A. C. Method (16)—Official

## 31.37

## REAGENT

*Petroleum benzine*.—Redistil below 75°. Make blank determination by evaporating 350 ml of the reagent with ca 0.25 g of stearine or other hard fat (previously brought to constant weight by heating) and drying as in actual determination. The blank must not exceed a few mg.

## 31.38

## APPARATUS

*Extraction cylinder*.—Glass-stoppered, graduated at 40 ml, 80 ml, and 130 ml, and of following dimensions: diameter ca 1½", height ca 12".

## 31.39

## DETERMINATION

Weigh 5 g ( $\pm 0.020$  g) of sample into a 200 ml Erlenmeyer flask, add 30 ml of redistilled alcohol and 5 ml of 50% aqueous KOH soln, and boil mixture for 1 hour under reflux condenser. Transfer to extraction cylinder and wash to 40 ml mark with redistilled alcohol. Complete transfer, first with warm, then with cold H<sub>2</sub>O, until total volume is 80 ml. Rinse flask with 50 ml of petroleum benzine and add rinsings to contents of cylinder previously cooled to room temp. Shake as vigorously as possible for 1 min. and allow to settle until both layers are clear, when volume of upper layer should be ca 40 ml. Draw off petroleum benzine layer as closely as possible by means of slender glass siphon into separator of 500 ml capacity. Repeat extraction at least 6 more times, using 50 ml of petroleum benzine for each extraction. Wash combined extracts in separator three times with 25 ml portions of 10% alcohol by volume, shaking vigorously each time. Transfer the petroleum benzine extract to weighed Erlenmeyer flask and distil; or, if desired, evaporate petroleum benzine on steam bath in current of air. Heat flask with residue in oven at uniform temp. not less than 100° nor more than 110° until constant weight is obtained. (A vacuum oven may be used at a corresponding temp., which depends upon pressure used in it. It is important to displace with air any residual vapors of petroleum benzine remaining in flask after heating and before it is weighed.) Deduct any blank from weight before calculating unsaponifiable matter. Test final residue for solubility in 50 ml of petroleum benzine at room temp. Filter, and wash free from insoluble residue, if any. Evaporate and dry in same manner as before.

## 31.40

## S. P. A. Method (17)—Tentative

Weigh accurately 2–2.5 g of fat into saponification flask (200 ml Erlenmeyer flask with 24/40 standard taper outer joint is recommended). Add 25 ml of alcohol and 1.5 ml of KOH soln (3 + 2). Saponify by boiling, with occasional swirling, on steam bath for 30 min. under reflux, air condenser. (No loss of alcohol should occur during saponification.) Transfer alcoholic soap soln while still warm to 250 ml separator, washing in with a total of 50 ml of H<sub>2</sub>O. Rinse saponification flask with 50 ml of ether and add the ether to contents of separator. Shake vigorously and allow layers to separate and clarify. Draw off lower layer and pour ether layer thru top into second separator containing 20 ml of H<sub>2</sub>O. Rinse pouring edge with ether, adding rinsings to second separator. Make two more extractions of the soap soln with 50 ml portions of ether in same manner.

Rotate combined ether extracts gently with the 20 ml of  $H_2O$  (violent shaking at this stage may result in troublesome emulsions). Allow layers to separate and draw off aqueous layer. Wash twice more with 20 ml portions of  $H_2O$ , shaking vigorously. Then wash alternately three times with 20 ml portions of ca 0.5  $N$  KOH and  $H_2O$ . If an emulsion forms during washing, draw off as much as possible of aqueous layer, leaving emulsion in separator with ether layer, and proceed with next washing. After third KOH treatment, wash ether soln successively with 20 ml portions of  $H_2O$  until washings are no longer alkaline to phenolphthalein.

Transfer ether soln to 250 ml lipped, conical beaker, rinsing separator and its pouring edge with ether and adding rinsings to main soln. Evaporate to ca 5 ml and transfer quantitatively, using several small portions of ether, to 50 ml fat or Erlenmeyer flask which has been previously dried and weighed, using similar flask as tare. Evaporate ether. When practically all ether has been removed, add 2 or 3 ml of acetone, and while heating on steam or water bath completely remove solvent in gentle current of air. Dry at  $100^\circ$  for 30 min. periods until weight is constant.

Dissolve contents of flask in 10 ml of freshly boiled, cooled, and neutralized (phenolphthalein) alcohol and titrate with 0.1  $N$  alcoholic NaOH (or KOH). (Not more than 0.10 ml is usually required.) Correct weight of residue for free fatty acid present (1 ml of 0.1  $N$  alkali = 0.0282 g of oleic acid).

Correct weight of residue for reagent blank obtained by conducting determination in same manner but omitting the fat.

#### SQUALENE (18)—TENTATIVE

##### 31.41

##### REAGENTS

(a) *Concentrated potassium hydroxide soln.*—Dissolve 60 g of KOH in 40 ml of  $H_2O$ .

(b) *Dilute potassium hydroxide soln.*—Dissolve 28 g of KOH in  $H_2O$  and dilute to 1 liter.

(c) *Petroleum benzine.*—Skellysolve B (b.p.  $63-70^\circ$ ) or equivalent.

(d) *Aluminum oxide adsorbent, 80-200 mesh.*—Adsorption alumina for chromatographic analysis, Fisher Scientific Co., Pittsburgh, Pa., or equivalent. Keep in tightly closed container, away from moisture.

(e) *Pyridine sulfate bromide soln.*—0.1  $N$ . Dissolve 8 g of Br in 20 ml of acetic acid (99.5%). Prepare another soln by adding gradually, with cooling, 5.45 ml of  $H_2SO_4$  to mixture of 20 ml of acetic acid and 8.15 ml of pyridine. Mix two solns, cool, and dilute to 1 liter with acetic acid.

(f) *Sodium thiosulfate soln.*—0.05  $N$ . Dissolve 13 g of  $Na_2S_2O_3 \cdot 5H_2O$  in  $CO_2$ -free  $H_2O$  containing 1% of iso-amyl alcohol, dilute to 1 liter, mix, and filter. Standardize against an exactly 0.05  $N$  soln of  $KIO_3$  (1.7835 g/liter) as follows: To glass-stoppered 125 ml Erlenmeyer flask, add 10 ml of 10% KI soln, 5 ml of  $H_2O$ , 2 g of  $NaHCO_3$ , and, slowly, 5 ml of ca 6  $N$  HCl. Mix, add 25 ml of the  $KIO_3$  soln, wash down sides of the flask with  $H_2O$ , and titrate at once with the  $Na_2S_2O_3$  soln, using starch indicator, 2.58(d), toward end of titration.

(g) *Potassium iodide soln.*—10%.

##### 31.42

##### APPARATUS

*Adsorption column.*—Prepare a fresh column for each determination immediately before use. Place small wad of cotton in constricted end of glass tube, 0.8 cm inside diam. and 30 cm long. Add  $Al_2O_3$  adsorbent in ca 10 small portions until column is ca 10 cm high. Apply gentle suction and tamp each portion of the  $Al_2O_3$  lightly with

flattened end of heavy glass rod. Place small wad of cotton on top of column and tamp lightly. Wash column with ca 15 ml of petroleum benzine, remove suction, and keep top of the column covered with shallow layer of petroleum benzine until ready for use.

## 31.43

## DETERMINATION

Weigh accurately ( $\pm 20$  mg) ca 5 g of sample into 125 ml Erlenmeyer flask, add 3 ml of the concentrated KOH soln and 20 ml of alcohol, and boil mixture under air condenser for 30 min., shaking flask occasionally. Cool somewhat, and while still warm, add 50 ml of petroleum benzine; mix, and transfer to separator. Rinse flask with 20 ml of alcohol and then with 40 ml of  $H_2O$ , adding rinsings to soln in separator. Shake vigorously, allow the two layers to separate completely, and slowly draw off soap soln. Pour the petroleum benzine extract from top of separator into another separator containing 20 ml of  $H_2O$ . Repeat extraction of soap soln with 50 ml of petroleum benzine. Rotate combined extracts gently with the 20 ml of  $H_2O$  and, after allowing layers to separate, discard wash water. Repeat washing by shaking vigorously with 20 ml of  $H_2O$  and again discard lower layer after separation. Wash petroleum benzine soln with 20 ml of the dilute KOH soln and then with successive 20 ml portions of  $H_2O$  until wash liquid is free from alkali, shaking vigorously on each occasion. After final washing, draw off last drops of  $H_2O$  brought down by swirling separator. Pour petroleum benzine soln from top of separator into lipped conical beaker. Rinse separator with petroleum benzine and add rinsings to beaker contents. Add few pieces of broken porcelain and evaporate almost all of solvent on steam bath. Remove last traces of solvent in current of  $CO_2$  or other inert gas, while warming beaker.

Dissolve the unsaponifiable matter in 5 ml of petroleum benzine and transfer to the adsorption tube. (The filtrate, which is caught in 250 ml glass-stoppered I flask, should emerge dropwise, at rate of ca 1 ml/min., gentle suction being used if necessary.) When soln has been nearly drawn into column, add ca 5 ml of the petroleum benzine that has been used to rinse beaker. Continue addition of solvent in 5–10 ml portions that have been used to rinse beaker, always keeping surface of column covered with liquid, until total volume of 50 ml has passed thru adsorption tube. Evaporate most of solvent in flask, after adding few pieces of broken porcelain, and remove last traces of solvent in atmosphere of  $CO_2$  or other inert gas.

Dissolve the unadsorbed residue in 5 ml of  $CHCl_3$  and add quantity of the pyridine sulfate bromide reagent sufficient to provide at least 50% excess (10 ml will usually be adequate). Allow mixture to remain in dark 5 min. and then add 5 ml of 10% KI soln, together with 40 ml of  $H_2O$ . Mix thoroly, wash down any free I on stopper, and titrate with the 0.05 N  $Na_2S_2O_3$ . Toward end of titration add the starch indicator, shake flask vigorously, and continue titration to disappearance of blue color. Conduct blank determination on the pyridine sulfate bromide reagent in same manner and calculate the ml of 0.05 N  $Na_2S_2O_3$  equivalent to absorbed halogen. Blank determination on all of reagents used should show practically no consumption of halogen. 1 ml of 0.05 N  $Na_2S_2O_3$  = 1.71 mg of squalene. Report results as mg of squalene/100 g of sample.

## ROSIN OIL

## 31.44

*Qualitative Test—Tentative*

Polarize the pure oil, or a definite dilution with petroleum benzine, in 200 mm tube. Rosin oil has a polarization in 200 mm tube of from  $+30^\circ$  to  $+40^\circ$  Ventzke, while most oils (19) read between  $+1^\circ$  and  $-1^\circ$ .

## COTTONSEED OIL

31.45

*Halphen Test (20)—Official*

Mix CS<sub>2</sub> containing 1% of S in soln with an equal volume of amyl alcohol. Mix equal volumes of this reagent and sample under examination and heat in bath of boiling, saturated NaCl soln 1–2 hours. In presence of as little as 1% cottonseed oil a pronounced characteristic red or orange-red color is produced. The depth of color is proportional, to a certain extent, to quantity of cottonseed oil present, and comparative tests with known mixtures of cottonseed oil will give an approximation of quantity.

Different oils react with different intensities. Oils that have been heated to 200–210° (21) react with greatly diminished intensity. Heating for 10 min. at 250° renders cottonseed oil incapable of giving the reaction (22). The fat of animals fed on cottonseed meal or other cottonseed products may give a positive reaction by this test.

## PEANUT OIL

31.46

*Modified Renard Test (23)—Official*

Weigh 20 g of the oil into Erlenmeyer flask. Saponify with alcoholic KOH soln, 31.24; neutralize exactly with acetic acid (1+3), using phenolphthalein indicator; and wash into an 800–1000 ml flask containing boiling mixture of 100 ml of H<sub>2</sub>O and 120 ml of 20% Pb acetate soln. Boil for a minute and then cool precipitated soap by immersing flask in H<sub>2</sub>O, occasionally giving it a whirling motion to cause soap to stick to sides of flask. After flask has cooled, decant the H<sub>2</sub>O and excess of Pb acetate soln and wash the Pb soap with cold H<sub>2</sub>O and alcohol, 90% by volume. Add 200 ml of ether, cork, and allow to stand until soap is disintegrated; heat on water bath, using reflux condenser, and boil ca 5 min. (24). In the case of oils, most of the soap will be dissolved, while in lards, which contain much stearin, part of soap will be left undissolved. Cool ether soln of soap to 15–17° and allow to stand until all insoluble soaps have separated out (ca 12 hours).

Filter upon Büchner funnel and thoroly wash insoluble Pb soaps with ether. Wash ether-insoluble Pb soaps into separator by means of jet of ether, alternating at end of operation if a little of the soap sticks to paper with HCl (1+3). Add sufficient HCl (1+3) so that total volume of acid amounts to ca 200 ml and enough ether to make its total volume 150–200 ml and shake vigorously for several minutes. Allow layers to separate, run off acid layer, and wash ether once with 100 ml of the dilute HCl and then with several portions of H<sub>2</sub>O until H<sub>2</sub>O washings are no longer acid to methyl orange. If a few undecomposed lumps of Pb soap remain (indicated by solid particles remaining after third washing with H<sub>2</sub>O), break these up by running off almost all H<sub>2</sub>O layer, adding a little HCl and shaking; then continue washing with H<sub>2</sub>O as before. Distil the ether from soln of insoluble fatty acids and dry latter in flask by adding a little absolute alcohol and evaporating on steam bath. Dissolve dry fatty acids by warming with 100 ml of 90% alcohol by volume. Cool slowly to 15°, shaking to aid crystallization. Allow to stand at 15° for 30 min. In presence of peanut oil, crystals of arachidic acid will separate from the soln. Filter, and wash precipitate twice with 10 ml of alcohol, 90% by volume, and then with alcohol, 70% by volume, taking care to maintain arachidic acid and wash solns at definite temp. in order to apply solubility corrections given below. Dissolve arachidic acid upon filter with boiling absolute alcohol, evaporate to dryness in weighed dish, dry, and weigh. Add to weight 0.0025 g for each 10 ml of 90% alcohol used in crystallization and washing, if conducted at 15°; if conducted at 20°, add 0.0045 g for each 10 ml. The melting point of arachidic acid thus obtained is 71–72°.



Twenty times weight of arachidic acid will give approximate quantity of peanut oil present. Arachidic acid has characteristic appearance and may be identified under microscope. As little as 5–10% of peanut oil can be detected by this method.

*Modified Bellier Test (25)—Tentative*

(Applicable only in presence of olive, cottonseed, corn, and soybean oils)

31.47

## REAGENTS

(a) *Alcoholic potassium hydroxide*.—1.5 *N*. Dissolve 10 g of KOH in purified alcohol, 31.24 (1), and make to 100 ml with purified alcohol.

(b) *Hydrochloric acid*.—Sp. gr. 1.16. Dilute 83 ml of the concentrated acid (sp. gr. 1.19) to 100 ml with H<sub>2</sub>O. Check with sp. gr. spindle.

(c) *Alcohol*.—70%. Dilute 700 ml of alcohol to 950 ml with H<sub>2</sub>O. Check by sp. gr. or refractive index and adjust if necessary.

31.48

## TEST

Weigh 0.92 g of the oil into 125 ml Erlenmeyer flask with standard taper outer joint, add 5 ml of the alcoholic KOH soln, and heat for 5 min. on steam bath, using air condenser to avoid loss of alcohol. Swirl once or twice during saponification. Add 50 ml of 70% alcohol and 0.8 ml of the HCl. Warm to dissolve any precipitate that may have formed. Cool soln in H<sub>2</sub>O, stirring continuously with thermometer so that temp. falls at rate of ca 1°/min. Observe turbidity temp. or clouding point, which is temp. at which a definite precipitate first appears. (It is essential that stirring be continuous and that the cooling H<sub>2</sub>O do not rise above level of liquid in flask since local cooling will cause premature formation of turbidity. Observe turbidity temp. by looking thru soln toward good light, or toward a dark background with good light coming from one side.)

If turbidity appears before temp. reaches 9° in case of olive oil, or 13° in case of cottonseed, corn, or soybean oils, presence of peanut oil is indicated. Confirm by 31.46.

31.49

## COLD TEST (26)—TENTATIVE

(Applicable to refined wintered salad oils)

Fill 4 oz. sample bottle with the oil at temp. of 25°, insert cork stopper tightly, and seal with paraffin. Submerge bottle completely in bucket containing finely cracked ice and add H<sub>2</sub>O until it rises to top of bottle. Keep bucket filled solidly with the ice by removing any excess H<sub>2</sub>O and adding ice when necessary. At end of 5 hours remove bottle and examine oil. If it is properly wintered, sample will be brilliant, clear, and limpid.

31.50

## TEA SEED OIL IN OLIVE OIL (27)—OFFICIAL

For preliminary qualitative test use following room temp. method: Measure into test tube (18×150 mm is convenient size) exactly 0.8 ml of acetic anhydride, 1.5 ml of CHCl<sub>3</sub>, and 0.2 ml of H<sub>2</sub>SO<sub>4</sub>. Mix, and cool to room temp. Add directly to reagents 7 drops of the oil to be tested, mix, and cool again. (To measure the 7 drops of oil use glass tubing, 4 mm outside diameter, and ca 2 mm inside diameter. These 7 drops should weigh ca 0.22 g.) If soln of oil in reagents is cloudy after mixing and cooling, add acetic anhydride dropwise, shaking after each addition until a clear

soln is suddenly formed. Appreciable deviations from these quantities, particularly in the  $\text{H}_2\text{SO}_4$ , cause distinct variations in color intensities. Since the mixed reagent deteriorates slowly do not mix in advance of testing.

After 5 min., add 10 ml of absolute ether from graduated cylinder and mix immediately by inverting once. Tea seed oil will show brown color changing to intense red within a minute or so. This red color reaches a maximum and then fades slowly within a few minutes. Olive oil forms an initial green color on addition of the ether. This color fades slowly to brown-gray, occasionally passing thru faint pink stage. Both olive oil and tea seed oil will eventually fade to a permanent light brown color. Mixtures of tea seed oil and olive oil show the characteristic tea seed oil colors proportional in intensity to quantity of tea seed oil present.

For approximately quantitative estimations drop oil into reagents as described above and allow to remain at room temp. for 5 min. In the meantime, cool 10 ml portion of absolute ether in ice water. At end of 5 min. period, place test tube containing oil and reagents in the ice water for 1 min., add the cold ether (taking care that no  $\text{H}_2\text{O}$  falls into test tube), and mix. Return tube to ice water bath and allow colors to develop while it is immersed in the ice water. The colors will develop slowly and reach a maximum within ca 5 min.

Use deepest red colors produced as basis for comparison, and because of short period of stable maximum intensity do not test more than three oils at one time. Standards containing known quantities of tea seed oil in an olive oil that gives little or no pink color with this test should be run simultaneously with sample. The preliminary room temp. test will give indication of standards to be used in the ice-water method.

#### SESAME OIL

31.51

##### *Modified Villavecchia Test (28)—Official*

Add 2 ml of furfural to 100 ml of alcohol. Mix thoroly 0.1 ml of this soln with 10 ml of  $\text{HCl}$  and 10 ml of the sample by shaking them together in test tube for 15 seconds. Allow mixture to stand for 10 min., observe color, add 10 ml of  $\text{H}_2\text{O}$ , shake, and again observe color. If the crimson color disappears, sesame oil is not present. (As furfural gives a violet tint with  $\text{HCl}$ , it is necessary to use the very dilute soln specified.)

31.52

##### FOREIGN FATS CONTAINING TRISTEARIN IN LARD (29)—TENTATIVE

Weigh 5 g of the melted and filtered lard into glass-stoppered cylinder and add 20 ml of warm acetone. Mix well, taking care that soln is clear and has temp. above  $30^\circ$ . Let stand at constant temp. of  $30^\circ$  for 16–18 hours. A fine mass of crystals occupying a volume of not more than 3 ml should then be found at bottom of cylinder. Should volume of crystals materially exceed 3 ml, take smaller quantity of lard (3–4 g) for new test. Should no crystals be deposited, as may be the case with soft or oily lard, absence of tristearin is indicated. Decant supernatant acetone soln from the crystallized glycerides. Add warm ( $30$ – $35^\circ$ ) acetone in three portions of 5 ml each from small wash bottle, taking care not to break up deposit in washing, and decant first two portions. Actively agitate third portion in the cylinder and by a quick movement transfer crystals to small filter paper. Using wash bottle, wash crystals with 5 successive small portions of the warm acetone and remove excess acetone by suction. Spread out the paper and its contents, breaking up any large lumps, and allow to dry in air at room temp. Thoroly comminute the mass and take melting point of crystals in a closed 1 mm tube, using apparatus similar to that

indicated under 31.14. Heat the  $H_2O$  in the beaker rapidly to ca  $55^\circ$  and maintain this temp. until thermometer carrying melting point tube registers  $50^\circ$ , then heat again and raise temp. of outer bath rather quickly to  $67^\circ$ . Remove burner. The melting point is reached when the fused substance becomes perfectly clear and transparent. When melting point of glycerides obtained by this method is below  $63.6^\circ$  the presence of beef fat or other fat containing tristearin should be suspected, and a melting point of  $63.2^\circ$  or lower is evidence that sample is not pure lard. It is advisable to carry out method with control sample of pure lard.

The conclusion indicated by melting point may be confirmed by taking melting point of the fatty acids prepared from the glycerides. After determining melting point, transfer crystallized glycerides to 50 ml beaker, add 25 ml of ca 0.5 *N* alcoholic KOH, and heat on steam bath until saponification is complete. Pour soln into separator containing 200 ml of  $H_2O$ , acidify, add 75 ml of ether, shake, and let stand. Draw off aqueous acid layer and wash ether soln at least 3 times with  $H_2O$ . Transfer ether soln to clean dry 50 ml beaker, volatilize ether on steam bath, and finally dry acids at  $100^\circ$ . Allow acids to remain at room temp. ca 2 hours and determine melting point. If the melting point of the glycerides, plus twice the difference between the melting point of the glycerides and the melting point of the fatty acids, is less than  $73^\circ$ , the lard is regarded as adulterated.

Conclusions may be confirmed further by precise determinations of the mean molecular weight of the separated fatty acids. Dissolve the acids in colorless, redistilled alcohol that has been carefully neutralized immediately before use, and titrate with 0.5–0.2 *N* KOH, using phenolphthalein indicator. Mean molecular weight = weight of fatty acids  $\times 1000 \div$  ml *N* KOH used. If sample is pure lard, the mean molecular weight of the fatty acids should correspond closely to that of the fatty acids of  $\alpha$ -palmito-distearin, 275.12. If sample is impure, the mean molecular weight should tend to approach that of the fatty acids from tristearin, 284.47.

#### FISH OIL AND MARINE ANIMAL OILS IN PRESENCE OF VEGETABLE OILS AND IN ABSENCE OF METALLIC SALTS

##### 31.53

##### *Qualitative Test—Tentative*

Using test tube dissolve ca 6 g of sample in 12 ml of mixture of equal parts of  $CHCl_3$  and acetic acid. Add Br, dropwise, until slight excess is indicated by color, keeping soln at ca  $20^\circ$ . Allow mixture to stand 15 min. or more and place test tube in boiling  $H_2O$ . If vegetable oils only are present, the soln will be perfectly clear, but fish oils will remain cloudy owing to presence of insoluble bromides.

#### MINERAL OIL IN FATS (50)

##### 31.54

##### *Qualitative Test—Official*

Place 1 ml of oil or melted fat in an Erlenmeyer flask, add 1 ml of KOH soln (3+2) and 25 ml of alcohol. Boil under reflux air condenser, shaking occasionally, until saponification is complete (ca 5 min.). Add 25 ml of  $H_2O$  and mix. In presence of more than 0.5% mineral oil, a distinct turbidity appears.

##### 31.55

##### *Quantitative Method—Official*

Treat the unsaponifiable matter, 31.39, with  $H_2SO_4$  as directed below. When very small quantities of mineral oil are present, sufficient unsaponifiable matter for the test may be obtained by following method: Saponify 100 g of fat by refluxing under air condenser for 2 hours with 55 ml of KOH soln (3+2) and 240 ml of alcohol, shaking flask occasionally. Cool, add 300 ml of petroleum benzine (b. p.  $35$ – $60^\circ$ )

and transfer to separator. Rinse flask with 240 ml of alcohol and add rinsings to separator. Add 480 ml of  $H_2O$  and shake vigorously. Allow layers to separate, withdraw lower layer, and transfer upper layer to another separator. Repeat extraction of saponified fat with 300 ml of petroleum benzine and combine extracts. Wash extract twice with 60 ml portions of  $H_2O$ , using gentle agitation. Repeat washing with 60 ml of 0.5 *N* KOH soln, followed by vigorous agitation with successive 60 ml portions of  $H_2O$  until washings are free from alkali. Evaporate extract to small volume and dry with anhydrous  $Na_2SO_4$ .

Filter petroleum benzine soln thru small plug of cotton into narrow-necked Babcock milk-test bottle, 22.26(a), add few small pieces of broken porcelain, and remove solvent by heating on steam bath while passing current of air thru bottle. Cool, add 5 ml of  $H_2SO_4$ , mix, and place bottle in boiling water bath for 30 min., shaking occasionally. Remove bottle from bath, cool, and fill with  $H_2SO_4$  until surface rises well into graduated neck. Centrifuge 5 min. at 1200 r.p.m. and read volume of unreacted residue. If sufficient mineral oil is available, obtain density as directed under 31.4, using small Sprengel tube. Weight of mineral oil can be closely approximated by multiplying volume by 0.88. Refractive index of colorless residue should be less than 1.500 at 20°.

#### COAL TAR COLORS (31)—TENTATIVE

##### 31.56

##### REAGENTS

(a) *Acid Soln A*.—Mix 1 liter of acetic acid with 200 ml of HCl and 100 ml of  $H_2O$ .

(b) *Acid Soln B*.—Cautiously add 400 ml of  $H_2SO_4$  to 100 ml of  $H_2O$ . When cool, add 900 ml of acetic acid and mix.

(c) *Sodium hydroxide soln*.—Approximately 25%. Dissolve 250 g of NaOH in  $H_2O$  and dilute to 1 liter.

##### 31.57

##### SEPARATION AND IDENTIFICATION

Place 125 ml of oil in each of six 500 ml separators and dilute each with 200 ml of low-boiling gasoline (b.p. 90–120°). Extract contents of all separators with same 50 ml portion of Acid Soln A, passing this soln thru each separator successively. Repeat this successive extraction 3 more times, using 50 ml of Acid Soln A each time. Transfer acid extract to flask containing 300 ml of the NaOH soln. Continue successive extractions of oil mixture, using two 15 ml portions of Acid Soln B, and combine these extracts with previous extracts. (Final aqueous extract should be alkaline to litmus.)

Cool, and extract 100 ml portions of this alkaline soln successively in two 250 ml separators, each containing 75 ml of petroleum benzine. Discard the clear, lower layers. Wash combined petroleum benzine extracts with 25 ml portions of  $H_2O$  until free from alkali and filter thru a folded filter. Presence of large quantity of chlorophyll is indicated by greenish residue on paper.

Wash the colored petroleum benzine extract with 25 ml portions of alcohol (70% by vol.) until lower layer is practically colorless, reserving petroleum benzine fraction for further tests. Alcohol extract may contain yellow AB (FD&C Yellow No. 3), yellow OB (FD&C Yellow No. 4), orange SS (FD&C Orange No. 2), aniline yellow (7) [15], butter yellow (16) [19], Sudan G (10) [23], Sudan I (11) [24]. Concentrate alcoholic extract in casserole to ca 50 ml, make slightly alkaline with  $NH_4OH$ , and dye color on washed silk strands until bath is exhausted. Compare color reactions produced on dyed fibers with various reagents with Table 1, Chap. 21.

Evaporate petroleum benzine fraction. If residue is greenish, dissolve in 15 ml of

petroleum benzine and extract with two 5 ml portions of Acid Soln B. Transfer acid extract to flask containing 50 ml of the NaOH soln, cool, and extract the alkaline soln in 20 ml portions with 25 ml of petroleum benzine. Wash benzine with H<sub>2</sub>O until free of alkali, and then with two 5 ml portions of 70% alcohol to remove traces of orange colors. Blue petroleum benzine soln at this stage indicates presence of quinizarine green SS (D&C Green No. 6). Evaporate petroleum benzine extract to dryness, dissolve residue in 10 ml of alcohol, make slightly alkaline with NH<sub>4</sub>OH, and dye on a few strands of washed silk to complete mechanical absorption.

### COTTONSEED (32)—OFFICIAL

(It is essential that each step in analysis of samples of cottonseed be executed promptly and with minimum exposure to oxidation. Once started, analytical operations should be continuous, with no interruption or delay at any point.)

#### SAMPLING

##### 31.58

##### EQUIPMENT

(a) *Trier*.—Corkscrew-type that will take ca 5 lbs. of cottonseed at a probe. Made of  $\frac{3}{4}$ " steel bent to form an open cylinder ca 4" in diam., the pitch of the twist being ca 2" and the screw portion being ca 34" long. It should not be turned after point reaches floor of car or truck, but withdrawn immediately.

(b) *Receptacle*.—Made by attaching an 8×5×5 $\frac{1}{2}$ " elevator bucket to a pole long enough to enable sampler to place bucket in level position near top of seed chute while standing outside of car and directly in front of it.

(c) *Shaker cleaner*.—Motor-driven. Has screens 3×7' and is provided with adjustable by-passes for securing a representative cross-section sample of seed for analysis.

(d) *Metal containers*.—2 $\frac{1}{2}$  bu. capacity, with close-fitting covers.

(e) *Cans or bags*.—Friction top cans, 155 cu. in. capacity, or 7 $\frac{1}{2}$ "×3"×14 $\frac{1}{2}$ " bags, 1/90 asphalt laminated, 1/60 duraloid sewn, open-mouth bottoms dipped in wax for sending samples to chemist.

(f) *Scales*.—Graduated in oz. or  $\frac{1}{2}$  oz.

(g) *Thermometer*.

##### 31.59

##### PROCEDURE

(a) *Car lots, before unloading*.—By means of trier, draw portions of the seed at different points in each end and in middle of car, taking, in all, not less than 10 portions. If car is so filled with seed that trier cannot be used, divide contents into 4 sections. In center of each section dig a hole 30" deep, using short-handled, 5-tined fork. From sides and bottom of each hole take ca 15 lbs. of seed with the fork. Place portion of seed taken at each hole in strong moisture-proof bag. Directly after sampling, collect and remove the four bags from car, empty into one of the metal sample containers, and replace cover.

(b) *Car lots, during unloading*.—Place the elevator bucket attached to a pole in center of unloading chute at regular intervals (depending upon rate of unloading) so that ca 2 lb. portions are taken for each ton of seed ejected from the car. (Whether drawn before or during unloading, the several portions collected from car lots should total not less than 50 lbs. in weight.)

(c) *Truck or wagon loads, before unloading*.—Insert trier in load at not less than 5 points. If load is so deep that trier does not reach bottom, fork the seed away from three different parts of load and with the trier withdraw three additional portions of

seed from bottom, making total of eight portions of seed. Weight of sample taken should not be less than 2 lbs. for each ton of seed in load.

If shipment of seed is hot at time of sampling, determine temp. with thermometer placed for not less than 5 min. at points near center of each end of car or near center of truck.

Immediately place each portion of original sample in properly identified metal container and cover promptly. Weigh entire sample and pass over a shaker cleaner. Collect and weigh all separated foreign matter or the cleaned seed. Calculate percentage of foreign matter.

If the shaker cleaner is not equipped with sample reducing device, place all cleaned seed in an efficient mixer (MacLellan Mixer, Model OCS, Serial No. 806). After mixing thoroly, discharge seed, and without any further mixing take 2 lb. sample for analysis, putting it without delay in air-tight container, 31.58(e). Also place inside can a report giving weights of original sample, cleaned seed, and foreign matter. Do all cleaning, mixing, and handling of samples expeditiously in order that they neither lose nor gain moisture.

**31.60****FOREIGN MATTER**

Examine laboratory sample immediately and if found not to have been thoroly cleaned carefully weigh and reclean by use of 6-mesh screen and by hand-picking of all remaining particles of foreign matter. Calculate percentage of foreign matter by dividing weight of foreign matter reported by sampler by weight of original sample and correcting result by adding percentage of foreign matter found in laboratory sample.

**31.61****MIXING AND QUARTERING**

Place sample in approved mechanical mixer (MacLellan Mixer No. 00-S) and mix according to directions for use of machine. After mixing, quarter sample and retain one-half in original container as referee sample. Preserve second half in air-tight container for analysis.

**31.62****MOISTURE (ORIGINAL)**

Weigh into shallow moisture dishes as rapidly as possible two portions of 8–10 g each of the whole seed and distribute evenly. Place uncovered dish in approved forced-draft circulating oven at 101° for 12–16 hours, or overnight. Remove dish from oven, cover, cool in efficient desiccator 30 min., and weigh. Calculate loss in weight as moisture.

**31.63****PREPARATION OF SEED FOR OIL AND AMMONIA DETERMINATIONS**

Dry an approximately 60 g portion for 2 hours at 130°  $\pm$  3°, in approved type of uniform forced-draft circulatory oven. Absorb 1.5 ml of HCl (for fuming delinted cottonseed use 1.0 ml HCl) into inner walls and bottom of porous earthenware vessel, such as 3" flower pot (unglazed porous clay pots made by Niloak Pottery Company of Benton, Arkansas, are satisfactory. The acid should be well distributed over sides of pot. When acid has been absorbed the pot should appear dry; if it does not it was probably not in proper condition for this use).

Cover pot containing dried seed with watch-glass and place in fuming oven (well-ventilated non-corrodible oven capable of reaching and maintaining temp. of 115°), previously opened and ventilated at least 5–10 min., and fume for 1 hour. (Oven temp. should gradually rise to, but not exceed, 115°. After fuming, lint should be loose and brittle but not scorched.) Grind treated seed in Bauer mill (No. 148 laboratory mill with No. 6912 plate), which has been adjusted to produce a fine meal.

After grinding, open mill and carefully brush out all remaining meal onto sizable smooth sheet of paper. (It is important that top of hopper of the Bauer mill be fitted with cover to prevent loss of seed during grinding.) If loss exceeds 1 g, repeat entire process as the remaining material is not necessarily representative of the whole.

Mix ground sample thoroly, preferably by placing it in 2 quart Mason fruit jar together with large rubber stopper. Replace cover and shake violently until ground material is thoroly mixed; transfer to well-stoppered bottle or container of just sufficient size to hold material tightly so as to prevent percolation or vertical segregation of the components.

## 31.64

## MOISTURE IN GROUND SAMPLE

Weigh 5 g of fumed and ground sample into moisture dish and dry at 101° for 2 hours in oven specified in 31.62. Calculate loss in weight as moisture content.

## OIL

## 31.65

## APPARATUS

(a) *Extractor*.—Butt type.

(b) *Condensers*.—Allihn with 12" jackets, fitted with cork connections.

## 31.66

## REAGENT

*Petroleum benzine*.—Initial boiling temp., 35–38°; dry-flask end point, 52–60°; at least 95% distilling under 54°, and not over 60% distilling under 40°; sp. gr. at 60°F., 0.630–0.660; color, water white; evaporation residue, not over 0.0011 g/100 ml; doctor test, sweet; copper-strip corrosion test, noncorrosive; trace only of unsaturated compounds permitted; residue in distilling flask, neutral to methyl orange; blotter strip odor test, odorless within 12 min.; aromatic compounds, no nitrobenzene odor; saponification value, less than 1.0 mg KOH per 100 ml.

Make distillation test according to method of American Society for Testing Materials (standard method D216–32) and make a blank by evaporating 250 ml with ca 0.25 g of stearin or other hard fat (previously brought to constant weight by heating) and drying as in actual determination. Blank must not exceed 3 mg.

## 31.67

## DETERMINATION

Weigh accurately duplicate samples of 4–5 g of the fumed and ground seed, 31.63, and wrap each portion in 150 mm filter paper (S & S No. 597 or equivalent grade); rewrap in second paper or papers in such manner as to prevent escape of the meal, leaving top of second paper open like a thimble. Place piece of absorbent cotton in top of thimble to distribute the dropping benzine. Place 25 ml of the petroleum benzine in tared flask, 125 ml capacity, and extract sample for 4 hours. (Benzine should drop on center of thimble at a rate of at least 150 drops per min., and volume of solvent should be kept ca constant.) Evaporate solvent until no trace remains, cool sample to room temp., and weigh. As last traces of benzine are sometimes difficult to detect by odor, in case of doubt heat for an hour, or longer, until constant weight is obtained. Calculate oil content as shown in following example:

Petroleum benzine extract.....	1.025 g
Original moisture plus total foreign matter up to and including 1.0%.....	12.2 + 0.8 = 13.0%
Total moisture.....	2.6%
Total foreign matter up to and including 1.0%.....	0.8%
Weight of sample.....	5.00 g

$$\frac{1.025}{5} \times \frac{87}{97.4} = 18.3\% \text{ of oil in original seed.}$$

## 31.68

## AMMONIA

Digest 1.7032 g of sample in 650–800 ml Kjeldahl flask with ca 0.5 g of Hg or 0.7 g of HgO, 10 g of Na<sub>2</sub>SO<sub>4</sub> or K<sub>2</sub>SO<sub>4</sub>, and 25 ml of H<sub>2</sub>SO<sub>4</sub> (sp. gr. 1.84). Place flask in inclined position and heat below boiling point of the acid from 5 to 15 min., or until frothing has ceased. Increase temp. and continue digestion until liquid becomes colorless, or until complete digestion is obtained.

After cooling mixture add ca 300 ml of H<sub>2</sub>O, a few granules of 20-mesh Zn to keep contents of flask from bumping, and 25 ml of 4% K<sub>2</sub>S or Na<sub>2</sub>S soln, or sufficient quantity to precipitate all the Hg. After mixing thoroly, add 60 ml of NaOH soln (sp. gr. 1.50), or sufficient to make strongly alkaline, pouring soln down side of flask so that it does not mix at once with the acid soln. Connect flask with condenser of block Sn, mix contents of flask by shaking, and distil into accurately measured quantity of standard H<sub>2</sub>SO<sub>4</sub> soln (0.5 N) to which has been added 50 ml of H<sub>2</sub>O, until at least 200 ml of distillate is obtained, taking care that delivery tube reaches below level of standard acid. Add ca 1 ml of 0.2% Na alizarin sulfonate soln as indicator. (Either cochineal or methyl red may be used as indicator, but with methyl red the soln is titrated hot.) Titrate distillate with standard 0.25 N NaOH soln.

By using 1.7032 g of sample for analysis, number of ml of 0.5 N acid required for neutralization of the distilled NH<sub>3</sub>, divided by 2, gives percentage of NH<sub>3</sub>.

Make blank test on all reagents and correct titration of above distillate accordingly. If percentage of NH<sub>3</sub> found in fumed, ground sample is less than 3.70%, or more than 4.50%, make second determination, and if these two determinations do not agree within 0.1%, make 2 additional determinations and use average of the 2 or 3 determinations agreeing most closely.

## 31.69

## CALCULATION

Example:

	ml
0.5 N H <sub>2</sub> SO <sub>4</sub> measured into flask.....	10.00
0.5 N H <sub>2</sub> SO <sub>4</sub> for blank test on reagents.....	0.06
0.25 N NaOH used in titration.....	2.68

$$\frac{10 - 0.06}{2} - \frac{2.68}{4} = 4.30\% \text{ ammonia in fumed seed.}$$

Original moisture.....	8.1
Moisture in fumed seed.....	2.0
Foreign matter, up to 1.0.....	0.9

$$\frac{4.30 \times 0.91}{0.98} = 3.99\% \text{ ammonia in original seed.}$$

## 31.70

## FREE FATTY ACIDS

Heat 200 g of original clean sample of seed for 30–40 min. at temp. of 100–105°, and cool. Pass the cooled seed thru an improved laboratory huller or Bauer mill set to merely crack all the seed. Separate meats from hulls by use of 4–6-mesh screen. Grind meats in Ruswine No. 1 food chopper equipped with 16-tooth blade. Thoroly mix sample. (Proper grinding and complete separation of meats from hulls are essential points in obtaining concordant results. All of the meats in a sample should be removed, and secured, ground, mixed, and quartered, since the oil in the last meats removed probably has less free fatty acids than the oil in the first meats removed. Further, all the oil in the sample of ground meats that may be used should be extracted, since the last oil extracted contains the higher percentages of free fatty acids.) Without undue loss of time quarter the thoroly mixed ground meats so as to obtain at least a 40 g sample. Extract this sample by cold percolation in follow-



ing manner: Place lower disk from Knorr extraction apparatus in Butt tube and place on it a layer of asbestos fiber suspended in petroleum benzine. (Satisfactory mat should allow none of meats to pass thru but should allow extracting solvent to flow thru at ca 150 drops/min.) Place sample in prepared tube, and add 50 ml of petroleum benzine followed by two portions of 25 ml each of petroleum benzine, allowing each portion to flow thru before adding next portion. Allow extracted oil to remain on steam bath for 1½ hours to completely remove all trace of solvent. Weigh 7.05 g of the oil into titration flask; add 30 ml of neutralized alcohol (SD Formula 30, 8.2) or isopropyl alcohol and 1 ml of 1% phenolphthalein soln (10 ml of petroleum benzine may be added if necessary); and titrate free fatty acids with 0.25 *N* alkali. Shake flask vigorously during titration, and take as end point a permanent pink that persists at least 1 min.

$$\% \text{ F.F.A.} = \frac{28.2 \times \text{normality of alkali} \times \text{ml used}}{\text{weight of oil}}$$

If results indicate free fatty acid content of 4% or higher, duplicate the complete test.

### FLAXSEED

#### OIL BY REFRACTION (53)—OFFICIAL

##### 31.71 PREPARATION OF STANDARD SOLVENT

Prepare a mixture of ca 74% halowax and 26%  $\alpha$ -bromonaphthalene by weight, and carefully adjust composition of mixture to refractive index of 1.63940 at 25.0°. (If a temp. regulating device is available the determination of refractive index is simplified by passing H<sub>2</sub>O at exactly 25.0° thru the water jacket of the refractometer. Equally satisfactory results may be obtained, however, by using H<sub>2</sub>O at room temp. and making necessary correction. For the above mixture this correction in refractive index is 0.00045 per 1°, to be added to reading if temp. is above 25.0° and subtracted if temp. is below that point. It is important that all water-jacket temp. readings be made to nearest 0.1°.)

Keep soln in glass or Pb-stoppered dark bottle and away from direct sunlight. (The refractive index should keep constant for a long period of time, but it is

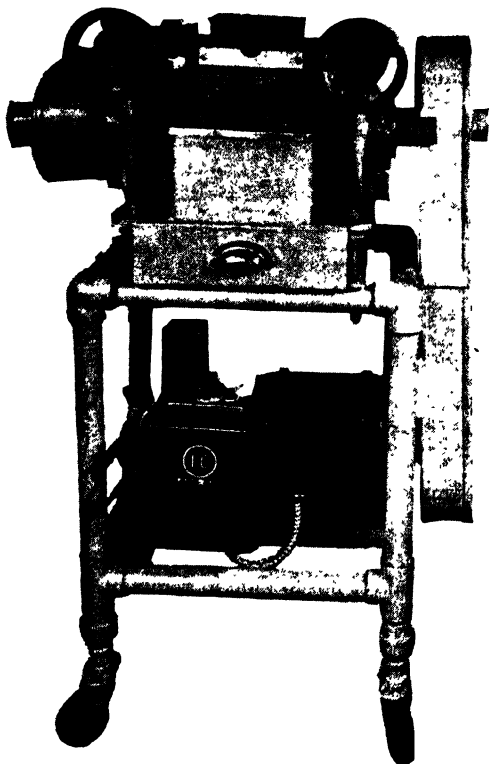


FIG. 51.—ROLLER-TYPE EXPERIMENTAL FLOURING MILL SUITABLE FOR GRINDING FLAXSEED SAMPLES

advisable to check it from time to time.)

## 31.72

## PREPARATION OF SAMPLE

Obtain representative sample of ca 25 g of the clean seed either by hand quartering original sample or by use of a mechanical sampling device. Grind material to such

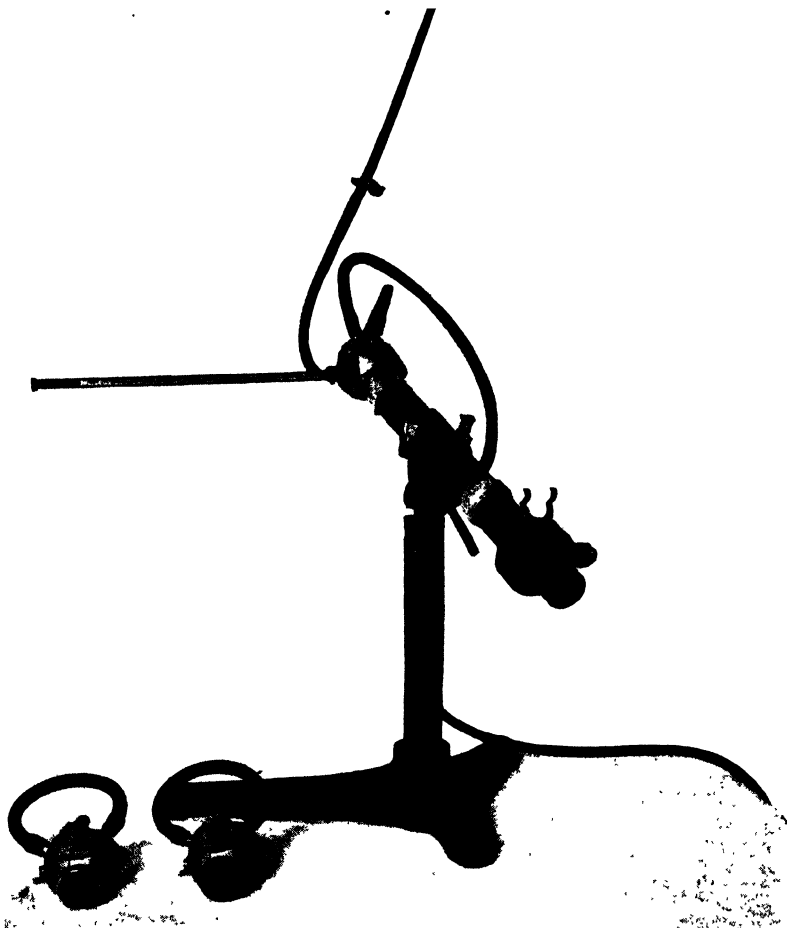


FIG. 52.—DIPPING-TYPE REFRACTOMETER WITH INTERCHANGEABLE DOUBLE PRISM HEADS SUITABLE FOR REFRACTOMETRIC DETERMINATION OF OIL CONTENT OF FLAXSEED

degree of fineness that after extraction with ether 95% of sample will pass thru 40-mesh sieve. (A motor-driven experimental roller flouring mill with 6×6" rolls, 40 corrugations to the inch, has been found satisfactory. The rolls should have a speed differential of 9:7 and the faster roll should have a speed of ca 900 r.p.m.) (See Fig. .51)

## 31.73

## DETERMINATION

Weigh out accurately 2.5 g of finely ground, well-mixed sample and transfer to

clean 3" porcelain mortar that has been previously heated to ca 70° in oven or on electric hot plate at low heat. Add ca 1 g of reagent quality sea sand or similar abrasive and exactly 5 ml of the standard mixture of halowax and  $\alpha$ -bromonaphthalene. (Since this mixture has a high sp. gr. it is important to measure its volume accurately. This is best accomplished with an accurately calibrated 5 ml pipet having delivery time of not less than 15 seconds.)

Grind mixture in mortar vigorously for 3 min., constantly scraping into bottom particles of meal that are thrown against sides of mortar. Filter mixture into test tube thru Schleicher and Schüll No. 588 folded paper, or other fat-free paper that will yield a clear filtrate. When filtrate has cooled to room temp., determine its refractive index at 25.0° to accuracy of  $\pm 0.00002$ . (Dipping-type refractometer equipped with interchangeable, water-jacketed, double prism heads is recommended, Fig. 52.) If reading is made at temp. other than 25.0°, make correction as directed in instructions for preparation of the standard solvent, using temp. coefficient of 0.00042 per 1°. Using table, 31.74, note percentage of oil corresponding to refractive index of filtrate (*uncorrected value* for oil content).

Place ca 2 g of the ground sample in fine paper filter in glass funnel and pour over it ca 15 ml of petroleum benzine, collecting clear filtrate in small shallow evaporating dish. Carefully evaporate off the ether on steam bath or hot plate at low heat, and place dish in oven at 105° for 20 min. Cool oil thus prepared to room temp. (If preferred, prepare this sample of oil by pressing small sample of the ground seed in a laboratory hydraulic press and filtering oil so obtained if it is not entirely clear.) Determine refractive index of the oil at 25.0°. The temp. coefficient for the pure oil is 0.000357 per 1.0°, to be added if temp. at which reading is taken is above 25.0° and subtracted if below that temp.

From the refractive index value of the oil subtract the value 1.47780 (refractive index at 25.0° of composite sample of oil used in obtaining data, 31.74). Using this difference, determine from the table, 31.75, the correction to be applied to the uncorrected value for oil content as determined above. If difference is positive, add correction; if negative, subtract.

**31.74** *Conversion table for determining percentage of oil in flaxseed from refractive index of the halowax,  $\alpha$ -bromonaphthalene extract at 25.0°*

$n_D^{25}$	Oil	$n_D^{25}$	Oil	$n_D^{25}$	Oil	$n_D^{25}$	Oil
	<i>per cent</i>		<i>per cent</i>		<i>per cent</i>		<i>per cent</i>
1.61837	28.0	1.61554	32.5	1.61279	37.0	1.61012	41.5
1.61831	28.1	1.61548	32.6	1.61273	37.1	1.61006	41.6
1.61824	28.2	1.61542	32.7	1.61267	37.2	1.61000	41.7
1.61818	28.3	1.61535	32.8	1.61261	37.3	1.60995	41.8
1.61811	28.4	1.61529	32.9	1.61255	37.4	1.60989	41.9
1.61805	28.5	1.61523	33.0	1.61249	37.5	1.60983	42.0
1.61799	28.6	1.61517	33.1	1.61243	37.6	1.60977	42.1
1.61792	28.7	1.61511	33.2	1.61237	37.7	1.60971	42.2
1.61786	28.8	1.61504	33.3	1.61231	37.8	1.60966	42.3
1.61779	28.9	1.61498	33.4	1.61225	37.9	1.60960	42.4
1.61773	29.0	1.61492	33.5	1.61219	38.0	1.60954	42.5
1.61767	29.1	1.61486	33.6	1.61213	38.1	1.60948	42.6
1.61760	29.2	1.61480	33.7	1.61207	38.2	1.60942	42.7
1.61754	29.3	1.61473	33.8	1.61201	38.3	1.60937	42.8
1.61748	29.4	1.61467	33.9	1.61195	38.4	1.60931	42.9
1.61742	29.5	1.61461	34.0	1.61189	38.5	1.60925	43.0
1.61735	29.6	1.61455	34.1	1.61183	38.6	1.60919	43.1
1.61729	29.7	1.61449	34.2	1.61177	38.7	1.60913	43.2
1.61723	29.8	1.61443	34.3	1.61171	38.8	1.60908	43.3
1.61716	29.9	1.61437	34.4	1.61165	38.9	1.60902	43.4
1.61710	30.0	1.61431	34.5	1.61159	39.0	1.60896	43.5
1.61704	30.1	1.61424	34.6	1.61153	39.1	1.60890	43.6
1.61697	30.2	1.61418	34.7	1.61147	39.2	1.60884	43.7
1.61691	30.3	1.61412	34.8	1.61141	39.3	1.60879	43.8
1.61685	30.4	1.61406	34.9	1.61135	39.4	1.60873	43.9
1.61679	30.5	1.61400	35.0	1.61130	39.5	1.60867	44.0
1.61672	30.6	1.61394	35.1	1.61124	39.6	1.60861	44.1
1.61666	30.7	1.61388	35.2	1.61118	39.7	1.60856	44.2
1.61660	30.8	1.61382	35.3	1.61112	39.8	1.60850	44.3
1.61653	30.9	1.61376	35.4	1.61106	39.9	1.60844	44.4
1.61647	31.0	1.61370	35.5	1.61100	40.0	1.60839	44.5
1.61641	31.1	1.61363	35.6	1.61094	40.1	1.60833	44.6
1.61635	31.2	1.61357	35.7	1.61088	40.2	1.60827	44.7
1.61628	31.3	1.61351	35.8	1.61082	40.3	1.60821	44.8
1.61622	31.4	1.61345	35.9	1.61076	40.4	1.60816	44.9
1.61616	31.5	1.61339	36.0	1.61071	40.5	1.60810	45.0
1.61610	31.6	1.61333	36.1	1.61065	40.6	1.60804	45.1
1.61604	31.7	1.61327	36.2	1.61059	40.7	1.60799	45.2
1.61597	31.8	1.61321	36.3	1.61053	40.8	1.60793	45.3
1.61591	31.9	1.61315	36.4	1.61047	40.9	1.60787	45.4
1.61585	32.0	1.61309	36.5	1.61041	41.0	1.60782	45.5
1.61579	32.1	1.61303	36.6	1.61035	41.1	1.60776	45.6
1.61573	32.2	1.61297	36.7	1.61029	41.2	1.60770	45.7
1.61566	32.3	1.61291	36.8	1.61024	41.3	1.60764	45.8
1.61560	32.4	1.61285	36.9	1.61018	41.4		

## 31.75 Corrections\* to be applied to results obtained in analysis of flaxseed for oil content by refractometric method

[Values to be added when  $(n_D^{25} - 1.4778)$  is positive, subtracted when  $(n_D^{25} - 1.4778)$  is negative]  
(Corrections in terms of per cent of oil indicated)

$n_D - 1.4778$	28°	29°	30°	31°	32°	33°	34°	35°	36°	37°	38°	39°	40°	41°	42°	43°	44°	45°	46°	47°	48°
0.0001...	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.03	0.03
0.0002...	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
0.0003...	0.05	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
0.0004...	0.06	0.06	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.09	0.09	0.09	0.09	0.10	0.10	0.10	0.10	0.10	0.10
0.0005...	0.08	0.08	0.08	0.09	0.09	0.09	0.10	0.10	0.10	0.11	0.11	0.11	0.11	0.11	0.12	0.12	0.12	0.12	0.12	0.12	0.12
0.0006...	0.09	0.10	0.10	0.10	0.11	0.11	0.11	0.12	0.12	0.12	0.13	0.13	0.13	0.13	0.14	0.14	0.14	0.14	0.14	0.14	0.14
0.0007...	0.11	0.11	0.12	0.12	0.12	0.13	0.13	0.14	0.14	0.15	0.15	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.17	0.17
0.0008...	0.12	0.13	0.13	0.14	0.14	0.15	0.15	0.16	0.16	0.17	0.17	0.18	0.18	0.18	0.19	0.19	0.19	0.19	0.19	0.19	0.19
0.0009...	0.14	0.14	0.15	0.15	0.16	0.16	0.17	0.17	0.18	0.18	0.19	0.20	0.20	0.20	0.21	0.21	0.21	0.21	0.21	0.21	0.21
0.0010...	0.15	0.16	0.17	0.17	0.18	0.18	0.19	0.20	0.20	0.21	0.22	0.22	0.23	0.23	0.24	0.24	0.24	0.24	0.24	0.24	0.24
0.0011...	0.17	0.17	0.18	0.19	0.20	0.20	0.21	0.22	0.22	0.23	0.24	0.25	0.25	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.27
0.0012...	0.18	0.19	0.20	0.21	0.22	0.22	0.23	0.24	0.24	0.25	0.26	0.27	0.27	0.28	0.28	0.29	0.29	0.29	0.29	0.29	0.29
0.0013...	0.20	0.21	0.22	0.22	0.23	0.24	0.25	0.26	0.26	0.27	0.28	0.29	0.30	0.31	0.31	0.32	0.32	0.32	0.32	0.32	0.32
0.0014...	0.21	0.22	0.23	0.24	0.25	0.26	0.27	0.28	0.28	0.29	0.30	0.31	0.32	0.32	0.33	0.33	0.33	0.33	0.33	0.33	0.33
0.0015...	0.23	0.24	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.35	0.36	0.36	0.36	0.36	0.36	0.36	0.36
0.0016...	0.24	0.25	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.37	0.38	0.38	0.38	0.38	0.38	0.38	0.38
0.0017...	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.40	0.40	0.40	0.40	0.40	0.40
0.0018...	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.42	0.42	0.42	0.42	0.42	0.42
0.0019...	0.29	0.30	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.44	0.44	0.44	0.44	0.44	0.44
0.0020...	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.45	0.45	0.45	0.45	0.45	0.45
0.0021...	0.32	0.33	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
0.0022...	0.34	0.35	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
0.0023...	0.35	0.37	0.38	0.40	0.41	0.43	0.44	0.45	0.46	0.47	0.48	0.50	0.51	0.53	0.54	0.55	0.55	0.55	0.55	0.55	0.55
0.0024...	0.37	0.38	0.40	0.41	0.43	0.44	0.46	0.47	0.49	0.50	0.52	0.54	0.55	0.57	0.59	0.61	0.62	0.64	0.65	0.66	0.66
0.0025...	0.38	0.40	0.42	0.43	0.45	0.46	0.48	0.49	0.51	0.53	0.55	0.58	0.60	0.61	0.63	0.64	0.65	0.66	0.67	0.68	0.68
0.0026...	0.40	0.41	0.43	0.45	0.46	0.48	0.50	0.51	0.53	0.55	0.57	0.60	0.62	0.64	0.65	0.66	0.67	0.68	0.69	0.70	0.70
0.0027...	0.41	0.43	0.45	0.46	0.48	0.50	0.52	0.53	0.55	0.57	0.59	0.62	0.64	0.66	0.68	0.69	0.71	0.72	0.72	0.72	0.72
0.0028...	0.43	0.45	0.46	0.48	0.50	0.52	0.53	0.55	0.57	0.59	0.61	0.63	0.65	0.68	0.70	0.72	0.74	0.76	0.77	0.77	0.77
0.0029...	0.44	0.46	0.48	0.50	0.52	0.54	0.55	0.57	0.59	0.61	0.63	0.65	0.66	0.68	0.70	0.72	0.74	0.76	0.77	0.79	0.81
0.0030...	0.46	0.48	0.50	0.52	0.53	0.55	0.57	0.59	0.61	0.63	0.65	0.67	0.69	0.71	0.73	0.74	0.76	0.78	0.80	0.82	0.84

\* Per cent oil as determined from 31.74.

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## 32. PRESERVATIVES AND ARTIFICIAL SWEETENERS

### SALICYLIC ACID

#### 32.1

#### PREPARATION OF SAMPLE—OFFICIAL

(a) *Non-alcoholic liquids*.—Many liquids may be extracted directly as described under 32.2 or 32.4 without further treatment. If troublesome emulsions form during extraction, pipet 100 ml into 250 ml volumetric flask, and add ca 5 g of NaCl, shaking until dissolved. Make up to mark with alcohol, shake vigorously, allow mixture to stand for 10 min. with occasional shaking, filter, and treat aliquot of filtrate as directed under (b).

(b) *Alcoholic liquids*.—Make 200 ml of sample alkaline with ca 10% NaOH soln, using litmus paper as indicator, and evaporate on steam bath to ca  $\frac{1}{2}$  its original volume. Dilute to original volume with H<sub>2</sub>O and filter if necessary.

(c) *Solid or semi-solid substances*.—Grind sample and mix thoroly. Transfer convenient quantity (50–200 g according to consistency of sample) to 500 ml volumetric flask, add sufficient H<sub>2</sub>O to make a volume of ca 400 ml, and shake until mixture becomes uniform. Add 2–5 g of CaCl<sub>2</sub> and shake until dissolved; render distinctly alkaline with ca 10% NaOH soln, using litmus paper as indicator; fill to mark with H<sub>2</sub>O, shake thoroly, allow to stand for at least 2 hours, shaking frequently, and filter.

#### QUALITATIVE TESTS

#### 32.2

#### Ferric Chloride Test—Official

Introduce 50 ml of sample or equivalent quantity of aqueous extract, prepared as directed under 32.1, into separator; add 1/10 its volume of HCl (1+3) and extract with 50 ml of ether. If mixture emulsifies, add 10–15 ml of petroleum benzine (b.p. below 60°) and shake. If this treatment fails to break emulsion, whirl mixture in centrifuge, or allow it to stand until considerable portion of aqueous layer has separated; run off latter, shake vigorously, and again allow to separate. Wash ether layer with two 5 ml portions of H<sub>2</sub>O, evaporate greater portion of the ether in porcelain dish on steam bath, allow remainder to evaporate spontaneously, and add one drop of 0.5% neutral FeCl<sub>3</sub> soln. A violet color indicates salicylic acid.

If coloring matter or other interfering substance is present in residue after evaporation of ether, purify the salicylic acid by one of following methods:

(a) Dissolve original residue from ether extract, obtained as directed above, in ca 25 ml of ether; transfer this soln to separator and shake with equal volume of H<sub>2</sub>O made distinctly alkaline with several drops of 10% NH<sub>4</sub>OH. Allow to separate, filter aqueous layer thru wet filter into porcelain dish, evaporate almost to dryness, and test residue as directed previously.

(b) Dry original residue from ether extract, obtained as directed above, in desiccator over H<sub>2</sub>SO<sub>4</sub> and extract with several 10 ml portions of CS<sub>2</sub> or petroleum benzine (b.p. below 60°), rubbing contents of dish with glass rod and filtering the successive portions of the solvent thru dry paper into a second porcelain dish. Evaporate the greater portion of solvent on steam bath, allow remainder to evaporate spontaneously, and test residue as directed previously.

(c) By means of a few ml of ether, transfer original residue from the ether extract, obtained as directed above, to small porcelain crucible, and allow solvent to evaporate spontaneously. Cut a hole in an asbestos board sufficiently large to admit ca  $\frac{1}{2}$  of crucible, cover with small, round-bottomed flask filled with cold H<sub>2</sub>O, and heat



over small Bunsen flame until any salicylic acid present has sublimed and condensed upon bottom of flask. Test sublimate as directed previously.

**32.3***Jorissen Test (1)—Official*

Dissolve residue from the ether extract, **32.2**, or, if impurities are present, the purified material obtained as directed under **32.2(a)**, (b), or (c), in a little hot  $\text{H}_2\text{O}$ . Cool 10 ml of soln in test tube; add 4 or 5 drops of 10%  $\text{KNO}_3$  soln, 4 or 5 drops of acetic acid (ca 50%), and 1 drop of 1%  $\text{CuSO}_4$  soln; mix thoroly, boil the liquid for half a minute, and allow to stand for 2 min. In presence of salicylic acid a Bordeaux-red color develops.

**QUANTITATIVE METHOD—OFFICIAL****32.4****EXTRACTION**

Transfer to separator 100 ml of sample, or that quantity of a soln prepared as directed under **32.1** which represents not less than 20 g of original material. If alkaline, neutralize to litmus with  $\text{HCl}$  (1+3) and add excess of  $\text{HCl}$  equivalent to 2 ml of acid for each 100 ml of soln. Extract with 4 separate portions of ether, using for each extraction a volume of ether equivalent to  $\frac{1}{4}$  the volume of aqueous layer. If emulsion forms on shaking, this may usually be broken by adding a little ( $\frac{1}{4}$  volume of ether layer) petroleum benzine (b.p. below  $60^\circ$ ) and shaking again, or by centrifuging. If small quantity of emulsion still persists, allow it to remain with aqueous layer, where frequently it is broken during next extraction. If an emulsion remains after fourth extraction, separate it from the clear ether and the clear aqueous layer and extract it separately with 2 or 3 small portions of ether. Combine ether extracts, wash with volume of  $\text{H}_2\text{O}$  equal to 1/10 of total volume of ether extracts, allow layers to separate, and reject aqueous layer. Wash in this way until aqueous layer after separation yields yellow color upon addition of methyl orange soln and 2 drops of 0.1  $N$   $\text{NaOH}$ . Distil slowly greater part of ether, transfer remainder to porcelain dish, and allow it to evaporate spontaneously. If no interfering substances are present, proceed as directed under **32.5**; if interfering substances are present, purify residue by one of following methods:

(a) Thoroly dry residue in vacuo over  $\text{H}_2\text{SO}_4$ . Extract it 10 times with 10–15 ml portions of  $\text{CS}_2$  or petroleum benzine (b.p. below  $60^\circ$ ), rubbing contents of dish with glass rod, and filter successive portions of solvent thru dry filter into porcelain dish. Test extracted residue with a drop of 2%  $\text{Fe}$  alum soln, and if it gives reaction for salicylic acid, dissolve it in  $\text{H}_2\text{O}$ ; acidify soln with  $\text{HCl}$  (1+3), extract with ether, evaporate, extract dry residue thus obtained with  $\text{CS}_2$  or petroleum benzine, and add to extract first obtained. Distil greater portion of the  $\text{CS}_2$  or petroleum benzine and allow remainder to evaporate spontaneously. Proceed as directed under **32.5**.

(b) Dissolve residue in 40–50 ml of ether. Transfer ether soln to a separator and extract with 3 successive 15 ml portions of 1%  $\text{NH}_4\text{OH}$ . (If fat is known to be present in original ether extract, extract latter directly with 4 portions of the  $\text{NH}_4\text{OH}$  instead of 3.) Combine alkaline aqueous extracts, acidify, again extract with ether, and wash combined ether extracts as directed previously. Slowly distil greater portion of the ether, allow remainder to evaporate spontaneously, and proceed as directed under **32.5**.

**32.5****DETERMINATION**

Dissolve residue, **32.4**, in small quantity of hot  $\text{H}_2\text{O}$ , and after cooling dilute to

definite volume (usually 50 or 100 ml). If soln is not clear, filter thru dry filter. Dilute aliquots of the soln and treat with 0.5%  $\text{FeCl}_3$  soln or 2% Fe alum soln until maximum color is developed. Generally a few drops will suffice.

(The Fe alum soln should be boiled until precipitate appears, allowed to settle, and filtered. Acidity of soln is slightly increased in this manner, but soln remains clear for a considerable time, and turbidity caused by its dilution with  $\text{H}_2\text{O}$  is much less and does not appear so soon as when unboiled soln is used. This turbidity interferes with exact matching of color.)

Compare colors developed with color obtained when a standard salicylic acid soln (containing 1 mg of salicylic acid in 50 ml) is similarly treated, using Nessler tubes or a colorimeter. In either case, and especially with  $\text{FeCl}_3$ , avoid excess reagent, altho an excess of 0.5 ml of 2% Fe alum soln may be added to 50 ml of the comparison soln of salicylic acid without vitiating results.

## BENZOIC ACID

### QUALITATIVE TESTS

#### 32.6

##### *Preliminary Test—Official*

Extract benzoic acid as directed under 32.2 or 32.4. If benzoic acid is present in considerable quantity, it will crystallize from the ether in shining leaflets having characteristic odor on warming. Dissolve crystalline deposit in hot  $\text{H}_2\text{O}$ , divide into 2 portions, and test as directed in 32.7 or 32.8. The deposit may also be purified as directed under 32.2(c) and the melting point determined.

#### 32.7

##### *Ferric Chloride Test—Official*

Make soln, 32.6, alkaline with a few drops of  $\text{NH}_4\text{OH}$ , expel excess of  $\text{NH}_3$  by evaporation, dissolve residue in few ml of hot  $\text{H}_2\text{O}$ , filter if necessary, and add a few drops of neutral 0.5%  $\text{FeCl}_3$  soln. A salmon-colored precipitate of ferric benzoate indicates presence of benzoic acid.

#### 32.8

##### *Modified Mohler Test (2)—Official*

(Presence of phenolphthalein interferes)

Add to the  $\text{H}_2\text{O}$  soln, 32.6, 1 or 2 drops of ca 10%  $\text{NaOH}$  soln and evaporate to dryness. To residue add 5–10 drops of  $\text{H}_2\text{SO}_4$  and a small crystal of  $\text{KNO}_3$ . Heat for 10 min. in bath (glycerol) at 120–130° (temp. must not exceed 130°). After cooling, add 1 ml of  $\text{H}_2\text{O}$  and make distinctly ammoniacal. Boil soln to decompose any  $\text{NH}_4\text{NO}_3$  that might have been formed. Cool, and add a drop of fresh, colorless  $(\text{NH}_4)_2\text{S}$  soln, but do not allow layers to mix. A red-brown ring indicates benzoic acid. On mixing the color diffuses thruout the liquid, and on heating finally changes to greenish yellow. This change differentiates benzoic acid from salicylic acid or cinnamic acid. The salicylic and cinnamic acids form colored compounds that are not destroyed by heating.

### QUANTITATIVE METHODS—OFFICIAL

#### 32.9

##### PREPARATION OF SAMPLE

(a) *General method.*—Mix sample thoroly, grinding if solid or semi-solid. Transfer 150 ml or 150 g to 500 ml volumetric flask, add enough pulverized  $\text{NaCl}$  to saturate the  $\text{H}_2\text{O}$  in sample, make alkaline to litmus paper with 10%  $\text{NaOH}$  soln or with milk

of lime (1 part powdered recently slaked  $\text{Ca}(\text{OH})_2$  suspended in 3 parts of  $\text{H}_2\text{O}$ ), and dilute to mark with saturated  $\text{NaCl}$  soln. Shake thoroly, allow to stand for at least 2 hours, with frequent shaking, and filter. If sample contains large quantities of fat, portions of which may contaminate a filtrate, add a few ml of the  $\text{NaOH}$  soln to filtrate and extract with ether before proceeding as directed under 32.10. If alcohol is present, proceed as directed under (d). If sample contains large quantities of matter precipitable by  $\text{NaCl}$  soln, proceed as directed under (e).

(b) *Ketchup*.—To 150 g of sample add 15 g of pulverized  $\text{NaCl}$ , and transfer mixture to 500 ml volumetric flask, rinsing with ca 150 ml of saturated  $\text{NaCl}$  soln. Make slightly alkaline to litmus paper with 10%  $\text{NaOH}$  soln and fill to mark with saturated  $\text{NaCl}$  soln. Allow to stand at least 2 hours, shaking frequently. Squeeze thru heavy muslin bag and filter.

(c) *Jellies, jams, preserves, and marmalades*.—Digest 150 g of sample in ca 300 ml of saturated  $\text{NaCl}$  soln. Add 15 g of pulverized  $\text{NaCl}$ . Make alkaline to litmus paper with milk of lime. Transfer to 500 ml volumetric flask and dilute to mark with saturated  $\text{NaCl}$  soln. Allow to stand at least 2 hours, shaking frequently; centrifuge if necessary, and filter.

(d) *Cider containing alcohol, and similar products*.—Make 250 ml of sample alkaline to litmus paper with 10%  $\text{NaOH}$  soln and evaporate on steam bath to ca 100 ml. Transfer sample to 250 ml volumetric flask, add 30 g of pulverized  $\text{NaCl}$ , and shake until dissolved. Dilute to original volume, 250 ml, with saturated  $\text{NaCl}$  soln; allow to stand at least 2 hours, shaking frequently, and filter.

(e) *Salted or dried fish*.—Wash 50 g of ground sample into 500 ml volumetric flask with  $\text{H}_2\text{O}$ . Make slightly alkaline to litmus paper with 10%  $\text{NaOH}$  soln and dilute to mark with  $\text{H}_2\text{O}$ . Allow to stand at least 2 hours, shaking frequently, and filter. Pipet as large a measured portion of filtrate as possible (at least 300 ml) into second 500 ml flask, and add 30 g of the pulverized  $\text{NaCl}$  for each 100 ml of soln. Shake until the  $\text{NaCl}$  has dissolved and dilute to mark with saturated  $\text{NaCl}$  soln. Mix thoroly and filter off precipitated protein and other extraneous matter.

### 32.10

#### DETERMINATION

Pipet a convenient portion (100–200 ml) of filtrate, 32.9, into separator. Neutralize to litmus paper with  $\text{HCl}$  (1+3) and add excess of 5 ml. In the case of salted fish a precipitation of protein matter usually occurs on acidifying, but precipitate does not interfere with the extraction. Extract carefully with  $\text{CHCl}_3$ , using successively portions of 70, 50, 40, and 30 ml. To avoid formation of an emulsion, shake cautiously each time, using rotary motion. The  $\text{CHCl}_3$  layer usually separates readily after standing a few minutes. If an emulsion forms, break it by stirring  $\text{CHCl}_3$  layer with glass rod, by drawing it off into second separator and giving one or two sharp shakes from one end of separator to other, or by centrifuging for a few minutes. As this is a progressive extraction, draw off carefully as much of the clear  $\text{CHCl}_3$  soln as possible after each extraction, but do not draw off any of emulsion with the  $\text{CHCl}_3$  layer. If this precaution is taken, the  $\text{CHCl}_3$  extract need not be washed.

Transfer combined  $\text{CHCl}_3$  extracts to porcelain evaporating dish, rinse container several times with a few ml of  $\text{CHCl}_3$ , and evaporate to dryness at room temp. in current of dry air.

The extract may also be transferred from the separator to a 300 ml Erlenmeyer flask, and the separator rinsed 3 times with 5–10 ml portions of  $\text{CHCl}_3$ . Distil very slowly at low temp. to ca  $\frac{1}{2}$  original volume. Transfer residue to porcelain evaporating dish, rinsing flask 3 times with 5–10 ml portions of  $\text{CHCl}_3$ , and evaporate to dryness at room temp. in current of dry air.

Dry residue overnight (or until no odor of acetic acid can be detected if the product is a ketchup) in desiccator containing  $\text{H}_2\text{SO}_4$ . Dissolve residue of benzoic acid in 30–50 ml of alcohol neutral to phenolphthalein; add ca  $\frac{1}{2}$  this volume of  $\text{H}_2\text{O}$  and 1 or 2 drops of phenolphthalein indicator, 2.10(d); and titrate with 0.05 *N* NaOH. 1 ml of 0.05 *N* NaOH = 0.0072 g of anhydrous Na benzoate.

## SACCHARIN

### QUALITATIVE TESTS

#### 32.11

#### *Organoleptic Test—Official*

Acidify with HCl 50 ml of a non-alcoholic liquid food or the aqueous extract of 50 g of a solid or semi-solid product, 32.14, and extract 3 times with 25 ml portions of ether. Wash combined ether extracts once with 5 ml of  $\text{H}_2\text{O}$ , transfer to small beaker or evaporating dish, allow ether to evaporate spontaneously, and taste residue. (Presence of as little as 20 mg of saccharin/liter or kg of original sample can usually be detected by its sweet taste.) Confirm by heating with NaOH and detecting the salicylic acid formed thereby as directed under 32.12.

#### 32.12

#### *By Conversion to Salicylic Acid—Official*

Acidify with HCl 50 ml of a non-alcoholic liquid food, or the equivalent quantity of aqueous extract, 32.14, and extract with 3 portions of ether as directed under 32.11. Dissolve residue remaining after evaporation of the ether in a little hot  $\text{H}_2\text{O}$  and test small portion of the soln for salicylic acid as directed under 32.2 or 32.3. Dilute remainder of soln to ca 10 ml and add 2 ml of  $\text{H}_2\text{SO}_4$  (1+3). Heat to boiling and add slight excess of 5%  $\text{KMnO}_4$  soln dropwise; partly cool soln, dissolve ca 1 g of NaOH in it, and filter mixture into an Ag dish (Ag crucible lids are well adapted to purpose). Evaporate to dryness and heat 20 min. at 210–215°. Dissolve residue in  $\text{H}_2\text{O}$ , acidify with HCl, and test ether extract for salicylic acid as directed under 32.2 or 32.3. By this method all so-called “false saccharin” (3) and any salicylic acid naturally present (also added salicylic acid when not present in too large a quantity) are destroyed, whereas 5 mg of saccharin/liter is detected with certainty.

#### 32.13

#### *Phenol-Sulfuric Acid Test (4)—Tentative*

(Applicable to non-alcoholic beverages, semi-solid preparations, and baked goods)

Prepare ethereal extract of sample as follows:

(a) *Non-alcoholic beverages*.—Add 3 ml of HCl to 25 ml of sample contained in separator. If vanillin is present, remove it by extracting with several portions of petroleum benzene. Discard petroleum benzene. Extract with 50, 25, and 25 ml of ether-petroleum benzene (1+1). Wash combined ethereal extracts once with 5 ml of  $\text{H}_2\text{O}$ , remove major portion of solvent, transfer to 30 ml beaker, and allow to dry at room temp.

(b) *Semi-solid preparations*.—Transfer 25 g of sample to 100 ml volumetric flask with small quantity of hot  $\text{H}_2\text{O}$  and add sufficient boiling  $\text{H}_2\text{O}$  to make ca 75 ml. Allow mixture to stand for an hour, shaking occasionally. Then add 3 ml of acetic acid, mix thoroly, add a slight excess (5 ml) of 20% neutral Pb acetate soln, dilute to mark with cold  $\text{H}_2\text{O}$ , mix, allow to stand for 20 min., and filter. Transfer 60 ml or more of the filtrate to separator and proceed as directed under (a).

(c) *Baked goods*.—Grind 25 g of sample, mix thoroly with 50 g of washed and ignited sea sand, and extract with petroleum benzene in a Soxhlet until approximately fat free (1–2 hours). Transfer extracted mass to 300 ml Erlenmeyer flask, add 100

ml of alcohol, and reflux on boiling water bath for 30 min., shaking frequently. Filter thru a Büchner funnel containing a Whatman No. 2, 7 cm filter paper wet with alcohol. Transfer the alcoholic filtrate to 100 ml beaker, evaporate to  $\frac{1}{2}$  volume, add 50 ml of  $H_2O$  and sufficient 10%  $Na_2CO_3$  soln to make alkaline, and evaporate to 50 ml. Transfer aqueous soln to separator and proceed as directed under (a).

To the residue remaining after evaporation of solvent add 5 ml of phenol- $H_2SO_4$  reagent (pure colorless crystalline phenol dissolved in equal weight of  $H_2SO_4$ ) and heat for 2 hours at 135–140°. Cool, dissolve in small quantity of hot  $H_2O$ , and pour into ca 250 ml of  $H_2O$ . Add small quantity of Filter-Cel, allow to stand 3 hours or overnight, and filter. Make alkaline with 10%  $NaOH$  soln and dilute to 500 ml. A magenta or reddish purple color develops if saccharin is present. A yellow, buff, or pale salmon shade is not significant.

#### QUANTITATIVE METHODS

##### I. General Method—Official

#### 32.14

##### PREPARATION OF SAMPLE

(a) *Fruit juices and sirups*.—Transfer 100–200 g of sample to 250 ml volumetric flask by means of a little  $H_2O$  and dilute to ca 200 ml with  $H_2O$ . Add 5 ml of acetic acid and mix. Add slight excess of 20% neutral Pb acetate soln, mix thoroly, dilute to mark with  $H_2O$ , again mix thoroly, and filter.

(b) *Alcoholic liquids*.—Heat 100–200 ml of liquid on steam bath to remove alcohol (usually done by evaporating to  $\frac{1}{2}$  original volume). With heavy sirups, dilute liquid with equal volume of  $H_2O$  before beginning evaporation. After alcohol has been removed, transfer liquid to 250 ml volumetric flask and proceed as directed under (a).

(c) *Solid or semi-solid preparations*.—Transfer 50–75 g of sample to 250 ml volumetric flask by means of a little hot  $H_2O$  and add sufficient boiling  $H_2O$  to make volume ca 200 ml. Allow mixture to stand for 2 hours, shaking occasionally. Add 5 ml of acetic acid, mix thoroly, add slight excess of 20% neutral Pb acetate soln, dilute to mark with cold  $H_2O$ , mix, allow to stand for 20 min., and filter.

#### 32.15

##### DETERMINATION

Transfer 150 ml of filtrate, 32.14, to a separator, add 15 ml of  $HCl$ , and extract 3 times with 80 ml portions of ether, shaking separator 2 min. each time. Wash combined ether extracts once with 5 ml of  $H_2O$ , remove ether by distillation, and transfer residue to Pt crucible by means of a little ether; or, if substances difficultly soluble in ether are present, use alternately small portions of  $H_2O$  and ether. Evaporate the ether on steam bath, add to residue 2–3 ml (or enough to make mixture strongly alkaline) of 10%  $Na_2CO_3$  soln, rotate so that all saccharin is brought in contact with the soln, and evaporate to dryness on steam bath. To dry residue in crucible add 4 g of a mixture of equal parts of anhydrous  $Na_2CO_3$  and  $K_2CO_3$ . Heat gently at first and then to complete fusion for 30 min. (Fusion may be conducted by closely fitting crucible into hole cut into piece of heavy asbestos board so that  $\frac{1}{2}$  of crucible projects above the asbestos, and heating lower portion of crucible by means of large Bunsen, Meker, or similar burner.) Cool, dissolve melt in  $H_2O$ , add ca 5 ml of Br water, acidify with  $HCl$ , filter, wash paper with a little  $H_2O$ , dilute filtrate and washings to ca 200 ml, heat to boiling, and slowly add excess of  $BaCl_2$  soln (ca 10%). Allow mixture to stand overnight, collect the  $BaSO_4$  on filter or on Gooch crucible, wash until free from chlorides, dry, ignite, cool, and weigh.

Correct result thus obtained for any S present in fusion mixture as found by blank determination. Calculate equivalent quantity of saccharin by multiplying corrected weight of  $\text{BaSO}_4$  by 0.7844.

(Instead of the mixed Na and K carbonates, 3–4 g of  $\text{Na}_2\text{O}_2$  may be used for the fusion. In this case a Ni crucible must be used, and time of fusion may be reduced to 5 min. The separation of a little  $\text{PbCl}_2$  during the extractions does not interfere with accuracy of method.)

**32.16 II. Special Method for Non-Alcoholic Beverages (5)—Official, First Action**

Add 2 ml of HCl to 50 ml of the drink contained in a separator. Extract with two successive 50 ml portions of ether. Filter ether extractions thru cotton, and wash combined filtrates with ca 5 ml of  $\text{H}_2\text{O}$  to which has been added 1 drop of HCl.

Separate ethereal layer and evaporate to dryness on water bath. Add to residue 5 ml of  $\text{NH}_3$ -free  $\text{H}_2\text{O}$  and 6 ml of HCl and evaporate soln to ca 1 ml on hot plate with constant stirring. Again add 5 ml of  $\text{NH}_3$ -free  $\text{H}_2\text{O}$  and 6 ml of HCl and evaporate to ca 1 ml. Dilute to 50 ml with  $\text{NH}_3$ -free  $\text{H}_2\text{O}$  and dilute 2 ml of this soln to 25 ml with  $\text{NH}_3$ -free  $\text{H}_2\text{O}$ . Add 1 ml of Nessler reagent, 37.10(a), and compare with  $\text{NH}_4\text{Cl}$  standards in usual manner; 0.2923 g of  $\text{NH}_4\text{Cl}$  = 1 g of saccharin, insoluble form, and = 1.317 g of the Na salt of the Pharmacopoeia crystallizing with 2 molecules of  $\text{H}_2\text{O}$  of hydration. For convenience prepare  $\text{NH}_4\text{Cl}$  standard equivalent to 200 p.p.m. of the insoluble form of saccharin.

## BORIC ACID AND BORATES

**32.17**

**QUALITATIVE TEST (6)—OFFICIAL**

(a) *Preliminary test.*—Acidity sample with HCl in proportion of 7 ml of acid to each 100 ml of sample. In case of solid or pasty samples heat with enough  $\text{H}_2\text{O}$  to make sufficiently fluid before acidifying. Immerse strip of turmeric paper in the acidified liquid, and allow the paper to dry spontaneously. If  $\text{Na}_2\text{B}_4\text{O}_7$  or  $\text{H}_3\text{BO}_3$  is present, the paper will acquire a characteristic red color, changed by  $\text{NH}_4\text{OH}$  to dark blue-green, but restored by acid.

(b) *Confirmatory test.*—Make ca 25 g of sample decidedly alkaline with lime  $\text{H}_2\text{O}$  or milk of lime and evaporate to dryness on steam bath. Ignite dry residue at low red heat until organic matter is thoroly charred. Cool, digest with ca 15 ml of  $\text{H}_2\text{O}$ , and add HCl dropwise until soln is distinctly acid. Immerse piece of turmeric paper in soln and allow to dry without heat. In presence of  $\text{Na}_2\text{B}_4\text{O}_7$  or  $\text{H}_3\text{BO}_3$ , the color change will be same as described under preliminary test.

**32.18**

**QUANTITATIVE METHOD (7)—OFFICIAL**

Make 10–100 g of sample (depending upon nature of material and quantity of  $\text{H}_3\text{BO}_3$  present) distinctly alkaline with NaOH soln and evaporate to dryness in Pt dish. Ignite residue until organic matter is thoroly charred, avoiding intense red heat; cool, digest with ca 20 ml of hot  $\text{H}_2\text{O}$  and add HCl dropwise until reaction is distinctly acid. Filter into 100 ml volumetric flask and wash with a little hot  $\text{H}_2\text{O}$ . (Volume of filtrate should not exceed 50–60 ml.) Return filter containing any unoxidized C to Pt dish, make alkaline by wetting thoroly with lime  $\text{H}_2\text{O}$ , dry on steam bath, and ignite to white ash. Dissolve ash in a few ml of HCl (1+3) and add to liquid in 100 ml flask, rinsing dish with a few ml of  $\text{H}_2\text{O}$ . To combined solns, add 0.5 g of  $\text{CaCl}_2$  and a few drops of phenolphthalein indicator, then 10% NaOH soln until a permanent light pink color is produced. Finally dilute to mark with lime  $\text{H}_2\text{O}$ , mix, and filter thru dry filter. To 50 ml of filtrate add 1 N  $\text{H}_2\text{SO}_4$ , 2.22(b),

until pink color disappears, then add methyl orange indicator, 6.3(f), and continue addition of the acid until yellow color is changed to pink. Boil ca 1 min. to expel  $\text{CO}_2$ . Cool, and carefully add 0.2 *N* NaOH until liquid assumes yellow tinge, avoiding excess of the alkali. (All the  $\text{H}_3\text{BO}_3$  is now in free state with no uncombined  $\text{H}_2\text{SO}_4$  present.) Add 1–2 g of neutral mannitol and a few drops of phenolphthalein indicator, note buret reading, and again titrate soln with the standard NaOH until pink color develops. Add a little more mannitol, and if pink color disappears continue addition of the standard alkali until pink color reappears. Repeat alternate addition of mannitol and standard alkali until a permanent end point is reached. A volume of glycerol (neutral to phenolphthalein) equal to volume of soln to be titrated may be substituted for the mannitol. 1 ml of 0.2 *N* NaOH soln = 0.0124 g of  $\text{H}_3\text{BO}_3$ .

### FORMALDEHYDE

#### 32.19

#### PREPARATION OF SAMPLE—OFFICIAL

If sample is solid or semi-solid, macerate 200–300 g with ca 100 ml of  $\text{H}_2\text{O}$  in mortar. Transfer to short-necked, 500–800 ml Cu or glass distillation flask, make distinctly acid with  $\text{H}_3\text{PO}_4$ , connect with condenser, and distil 40–50 ml. With highly colored liquids, make ca 200 ml distinctly acid with  $\text{H}_3\text{PO}_4$  and distil as directed previously.

#### QUALITATIVE TESTS

#### 32.20

#### *I. Phenylhydrazine Hydrochloride Test (8)—Official*

(Gives reliable reactions for  $\text{HCHO}$  in soln varying from 1 part in 50,000 to 1 part in 150,000. Neither acetaldehyde nor benzaldehyde interferes with reaction.)

With milk and other liquids, shake with an equal volume of alcohol, filter and use filtrate. With meats and fats, extract the  $\text{HCHO}$  with alcohol and use filtrate. With fat, heat mixture above melting point of the fat to insure thoro extraction.

Mix 5 ml of distillate obtained under 32.19, or of an alcoholic soln or extract, obtained as directed previously, with 0.03 g of phenylhydrazine hydrochloride and 4 or 5 drops of a 1%  $\text{FeCl}_3$  soln. Add 1–2 ml of  $\text{H}_2\text{SO}_4$  slowly and with agitation, keeping the liquid in bath of cold  $\text{H}_2\text{O}$  to prevent heating. Dissolve precipitate by addition of either  $\text{H}_2\text{SO}_4$  (keeping mixture cool) or alcohol. In presence of  $\text{HCHO}$  a red color develops.

#### 32.21

#### *II. Hehner Test (9)—Official*

Mix in test tube ca 5 ml of distillate obtained under 32.19 with an equal volume of pure milk or with 1–2% soln of egg albumin, and underlay with commercial  $\text{H}_2\text{SO}_4$  without mixing. A violet or blue color at junction of the two liquids indicates  $\text{HCHO}$ . This color is given only in presence of trace of  $\text{FeCl}_3$  or other oxidizing agent. If only pure acid is available, add a few drops of  $\text{FeCl}_3$  soln to acid before making test. Milk may be treated directly by this method, and it gives positive tests in presence of one or more parts of  $\text{HCHO}/10,000$ . Other articles of food rich in proteins, for example, egg albumin, give the reaction in the presence of  $\text{H}_2\text{O}$  without addition of milk.

#### 32.22

#### *III. Leach Test—Official*

Mix in porcelain casserole ca 5 ml of distillate, 32.19, with an equal volume of pure milk and add ca 10 ml of  $\text{HCl}$  soln containing 2 ml of 10%  $\text{FeCl}_3$  soln to each liter

of acid. Heat to 80–90° directly over gas flame, rotating casserole to break up the curd. A violet coloration indicates HCHO.

**32.23**      *IV. Phenylhydrazine Hydrochloride and Sodium Nitroprusside*  
*Test (10)—Official*

(Applicable directly to liquid foods, to an aqueous or alcoholic extract of solid foods, or to distillate prepared as directed under 32.19)

In the case of milk, apply method directly. With meat, comminute sample, extract with 2 volumes of hot  $H_2O$ , and use expressed liquid for test. Heat ca 10 g of fats above their melting point with 20 ml of alcohol, shake thoroly, cool, filter thru moistened filter, and use filtrate for test.

Dissolve a lump of phenylhydrazine hydrochloride about size of a pea in 3–5 ml of liquid to be tested, and add 2–4 drops (not more) of 5–10% Na nitroprusside soln and 8–12 drops of ca 10% NaOH soln. If HCHO is present, a green or blue color, depending upon quantity, develops. When HCHO is present to extent of more than 1 part in 70,000–80,000 in soln tested, a distinct green or bluish green coloration is obtained. In more dilute solns the green tint becomes less marked, and a yellow tinge tending toward greenish brown develops. With this test acetaldehyde and benzaldehyde give a color varying, according to strength of the soln, from red to brown. Therefore, a reaction may be obtained with these aldehydes similar to that obtained with HCHO in solns more dilute than 1 part in 70,000. The presence of acetaldehyde or benzaldehyde together with HCHO gives a yellowish or yellowish green tinge. The reaction for HCHO, therefore, may be masked by presence of other aldehydes, but it is characteristic when a clear green color is obtained.

**32.24**      *V. Phenylhydrazine Hydrochloride and Potassium Ferri-*  
*cyanide Test (10)—Official*

(Not applicable in presence of coloring matter of blood)

Proceed as directed under 32.23, substituting a soln of  $K_3Fe(CN)_6$  for the Na nitroprusside. HCHO gives a red color. Alcoholic extracts from foods must be diluted with  $H_2O$  to prevent precipitation of  $K_3Fe(CN)_6$ .

**32.25**      *VI. Phenylhydrazine Hydrochloride and Ferric Chloride Test (10)—Official*

Treat 15 ml of milk or other liquid food, or of distillate prepared as directed under 32.19, with 1 ml of 1% phenylhydrazine hydrochloride soln, then with a few drops of 1%  $FeCl_3$  soln, and finally with HCl. The presence of HCHO is indicated by the formation of red color, which changes after some time to orange yellow. Milk may be examined directly by this method, but more delicate tests may be obtained from distillate from milk or from milk serum. Acetaldehyde or benzaldehyde does not interfere with the reaction.

**32.26**      *VII. Phloroglucinol Test (11)—Official*

To 10 ml of milk or other liquid food under examination in test tube add, by means of pipet, 2 ml of phloroglucinol reagent (1 g of phloroglucinol, 20 g of NaOH, and  $H_2O$  to make 100 ml), placing end of pipet on bottom of tube in such a manner that the reagent will form a separate layer. If HCHO is present, a bright red coloration (not purple) forms at zone of contact.

(This soln gives yellow color in presence of some aldehydes, and if it is used for detection of aldehyde formed by oxidation of methyl alcohol after the destruction of



acetaldehyde with  $\text{H}_2\text{O}_2$  soln, orange yellow color will slowly appear when insufficient quantity of  $\text{H}_2\text{O}_2$  soln has been used. On the other hand, if excess of  $\text{H}_2\text{O}_2$  soln is not fully destroyed before use of this reagent, a purple color develops slowly. The clear red color given by this reagent forms quickly, and in presence of but a small quantity of  $\text{HCHO}$  it fades rapidly.)

### SOLUBLE FLUORIDES

#### 32.27

#### QUALITATIVE TESTS (18)—OFFICIAL

(a) *Not applicable in presence of silicates.*—After thoroly mixing sample transfer to beaker 150 ml, or an equivalent quantity of aqueous extract in the case of solid foods, and boil, adding 5 ml of 10%  $\text{K}_2\text{SO}_4$  soln and 10 ml of 10% Ba acetate soln. Collect precipitate in compact mass (centrifuge may be used advantageously) and wash upon small filter. Transfer to Pt crucible and ignite.

Dip a carefully cleaned glass plate, while hot, in mixture of equal parts of carnauba wax and paraffin and allow to cool. Make a distinctive mark thru the wax with sharp instrument, taking care not to scratch surface of glass.

Add a few drops of  $\text{H}_2\text{SO}_4$  to residue in crucible and cover crucible with the waxed plate, having mark over center of crucible and making sure that edge of crucible is in close contact with plate. Keep top surface of plate cool by means of suitable device and heat crucible for an hour at as high a temp. as practicable without melting wax (electric stove gives most satisfactory form of heat). If fluorides are present, a distinct etching will be apparent on glass where it was exposed.

(b) *Applicable in presence of silicates.*—Test (a) may be varied by mixing small quantity of precipitated  $\text{SiO}_2$  with the precipitated  $\text{BaF}_2$  and applying method for detection of fluosilicates, 32.29 or 32.30.

This method is of value in the case of foods, the ash of which contains considerable quantity of  $\text{SiO}_2$ . Under these circumstances  $\text{H}_2\text{SO}_4$  liberates  $\text{SiF}_4$ , which would escape detection under (a).

### INSOLUBLE FLUORIDES

(Fluoborates, fluosilicates, etc.)

#### 32.28

#### PREPARATION OF SAMPLE—OFFICIAL

Make ca 200 g of sample alkaline with lime  $\text{H}_2\text{O}$ , evaporate to dryness, and incinerate. Extract crude ash with  $\text{H}_2\text{O}$ , to which has been added sufficient acetic acid to decompose carbonates; filter, ignite insoluble portion, extract with acetic acid (1+2), and again filter. Insoluble portion now contains  $\text{CaSiO}_3$  and  $\text{CaF}_2$ , while filtrate contains all the  $\text{H}_3\text{BO}_3$  present.

#### 32.29

#### QUALITATIVE TEST I (19)—OFFICIAL

Incinerate filter containing insoluble portion from 32.28, mix with a little precipitated  $\text{SiO}_2$ , transfer to short test tube attached to small U-tube containing a few drops of  $\text{H}_2\text{O}$ , and add 1–2 ml of  $\text{H}_2\text{SO}_4$ . Keep test tube in beaker of  $\text{H}_2\text{O}$  on steam bath for 30–40 min. If any F is present, the  $\text{SiF}_4$  generated will be decomposed by the  $\text{H}_2\text{O}$  in U-tube and will form a gelatinous deposit on walls of tube. Test filtrate for  $\text{H}_3\text{BO}_3$  as directed under 32.17. If both HF and  $\text{H}_3\text{BO}_3$  are present, it is probable that they are combined as  $\text{BF}_3$ . If, however,  $\text{SiF}_4$  is detected and  $\text{H}_3\text{BO}_3$  is not, repeat test without introducing the  $\text{SiO}_2$ , in which case formation of the  $\text{SiO}_2$  skeleton is conclusive evidence of presence of fluosilicate. In an ash containing an appreciable quantity of  $\text{SiO}_2$ ,  $\text{H}_2\text{SO}_4$  will liberate  $\text{SiF}_4$  rather than HF. Therefore the presence of a fluosilicate, not a fluoride, is indicated.

## 32.30

## QUALITATIVE TEST II—OFFICIAL

Incinerate filter containing insoluble portion from 32.28 in Pt crucible, mix with a little precipitated  $\text{SiO}_2$ , and add 1 ml of  $\text{H}_2\text{SO}_4$ . Cover crucible with watch-glass from underside of which a drop of  $\text{H}_2\text{O}$  is suspended, and heat for an hour at  $70\text{--}80^\circ$ , keeping watch-glass well cooled. The  $\text{H}_2\text{O}$  decomposes the  $\text{SiF}_4$  which is formed, leaving gelatinous deposit of  $\text{SiO}_2$  and etching a ring at periphery of drop of  $\text{H}_2\text{O}$ . Test filtrate for  $\text{H}_2\text{BO}_3$  as directed under 32.17.

## SULFUROUS ACID

## 32.31

## QUALITATIVE TEST (14)—OFFICIAL

Add a small quantity of S-free Zn and several ml of HCl to ca 25 g of the sample (with addition of  $\text{H}_2\text{O}$ , if necessary) in 200 ml Erlenmeyer flask. The  $\text{H}_2\text{S}$  generated in presence of sulfites may be detected with Pb acetate paper. The traces of metallic sulfides occasionally present in vegetables will give the same reaction as sulfites under the conditions of the above test. Verify positive results obtained by this method by the Monier-Williams method, 32.32.

It is always advisable to make the quantitative determination of sulfites, owing to the danger to the test caused by traces of sulfides. A trace should not be considered sufficient indication of the presence of  $\text{SO}_2$  either as a bleaching agent or as a preservative.

## TOTAL SULFUROUS ACID

## 32.32

*Monier-Williams Method (15)—Official*

(Applicable in presence of other volatile S compounds)

Connect 750 ml round-bottomed Pyrex flask (B) (Fig. 53) to sloping reflux condenser (D), lower end of which is cut off at an angle. (Monier-Williams prefers using upright round-bottomed 1500 ml flask with 2 necks.) Pass  $\text{CO}_2$  from a generator thru a  $\text{Na}_2\text{CO}_3$  soln in A to remove Cl. Also connect a dropping funnel (K) to B by three-holed stopper C. Use tube E to connect upper end of condenser to 200 ml Erlenmeyer flask (F), which is followed by a Peligot tube (G). This delivery tube (E) extends to bottom of receiver. One Peligot tube has been found to be sufficient to catch traces of sulfurous acid swept thru flask F. Use rubber stoppers thruout. The receiver F contains 15 ml of pure neutral 3%  $\text{H}_2\text{O}_2$ , while the Peligot tube contains 5 ml.  $\text{H}_2\text{O}_2$  usually contains free  $\text{H}_2\text{SO}_4$ . Start with 30%  $\text{H}_2\text{O}_2$ , dilute somewhat, and neutralize with  $\text{Ba}(\text{OH})_2$  soln, using bromophenol blue soln as indicator. After the reagent has settled in the cold, filter from the  $\text{BaSO}_4$ , determine its concn by  $\text{KMnO}_4$  titration, and finally adjust to 3% concn. The bromophenol blue indicator in the  $\text{H}_2\text{O}_2$  remains unaffected for some time.

After connecting apparatus, introduce into flask 300 ml of  $\text{H}_2\text{O}$  and 20 ml of HCl and boil for short time in current of  $\text{CO}_2$ . Add food to be tested, adapting procedure to sort of food. Add liquids directly by means of the dropping funnel. In case of easily transferable solids, first cool contents of flask somewhat, taking care to regulate flow of  $\text{CO}_2$  to avoid having the  $\text{H}_2\text{O}_2$  drawn up in delivery tube E. Then quickly introduce the food by removing stopper C. (Solid foods such as meat and ground dry fruits may be quickly introduced by first wrapping in filter paper.) With semi-solid foods, requiring more time to introduce into the flask, cool flask contents by gradual immersion in cold  $\text{H}_2\text{O}$ , and wash the food in quickly with recently boiled  $\text{H}_2\text{O}$ . After introducing the food, boil mixture for 1 hour ( $1\frac{1}{2}$  hours in

the case of dried fruits) in slow current of  $\text{CO}_2$ , stopping flow of  $\text{H}_2\text{O}$  in condenser just before the end of distillation. This causes condenser to become hot and drives over residual traces of  $\text{SO}_2$  retained in condenser. When the delivery tube just above the receiver E becomes hot to the touch, remove stopper J immediately.

Wash delivery tube and the Peligot tube contents into flask F, and titrate liquid at room temp. with 0.1 N NaOH, using bromophenol blue as indicator. The NaOH must be standardized with this indicator. Bromophenol blue is unaffected by  $\text{CO}_2$ , and also gives a distinct color change in cold  $\text{H}_2\text{O}_2$ . 1 ml of 0.1 N NaOH = 3.2 mg

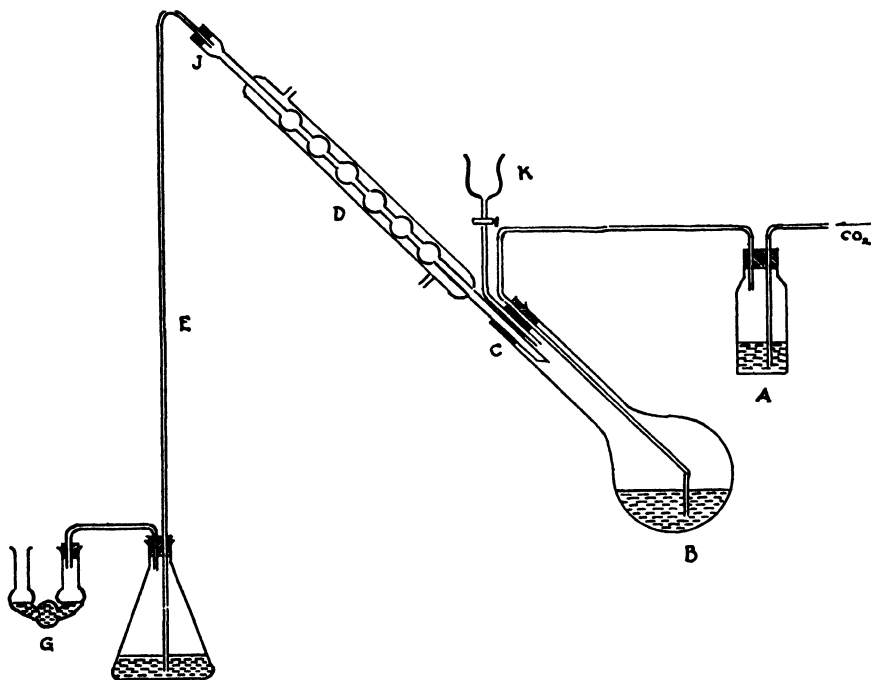


FIG. 53.—MONIER-WILLIAMS APPARATUS FOR DETERMINATION OF SULFUROUS ACID

of  $\text{SO}_2$ , so that titration of small quantities of  $\text{SO}_2$  requiring less than 0.5 ml of NaOH is not accurate. A gravimetric determination may be made after titration, the precipitation of  $\text{BaSO}_4$  being carried out at room temp. After allowing the supernatant liquid to settle, filter, and wash residual  $\text{BaSO}_4$  3 times by decantation with boiling  $\text{H}_2\text{O}$ . Determine blank on reagents, both by titration and gravimetrically, and correct results accordingly.

### 32.33

#### FREE SULFUROUS ACID—OFFICIAL

Treat 50 ml of sample in 200 ml flask with ca 5 ml of  $\text{H}_2\text{SO}_4$  (1+3), add ca 0.5 g of  $\text{Na}_2\text{CO}_3$  to expel air, and titrate  $\text{H}_2\text{SO}_4$  with 0.02 N I soln, using a few ml of starch indicator, 6.3(e). Introduce the I soln as rapidly as possible and continue addition until the blue color persists for several minutes. 1 ml of 0.02 N I = 0.64 mg of  $\text{SO}_2$ .

**BETA-NAPHTHOL****32.34****QUALITATIVE TEST—TENTATIVE**

In a separator extract 200 ml of the sample or of its aqueous extract, prepared as directed under 32.1(c), with 10 ml of  $\text{CHCl}_3$ . To the  $\text{CHCl}_3$  extract in test tube add a few drops of 0.5 *N* KOH soln and place in boiling water bath for 2 min. Presence of  $\beta$ -naphthol is indicated by formation of deep blue color, which changes to green and then to yellow.

**ABRASTOL (ASAPROL)****32.35****I. SINIBALDI METHOD (16)—TENTATIVE**

Make 50 ml of the sample alkaline with a few drops of  $\text{NH}_4\text{OH}$  and extract with 10 ml of amyl alcohol, adding ethyl alcohol if an emulsion forms. Decant the amyl alcohol, filter if turbid, and evaporate to dryness. Add to residue 2 ml of  $\text{HNO}_3$  (1+1), heat on water bath until half of liquid is evaporated, and transfer to test tube with addition of 1 ml of  $\text{H}_2\text{O}$ . Add ca 0.2 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  and an excess of  $\text{NH}_4\text{OH}$ , dropwise, with constant shaking. If resultant precipitate is of reddish color, dissolve it in a few drops of  $\text{H}_2\text{SO}_4$ , and add  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{NH}_4\text{OH}$  as before. As soon as a dark colored or greenish precipitate is obtained, introduce 5 ml of alcohol, dissolve precipitate in  $\text{H}_2\text{SO}_4$ , shake well, and filter. In the absence of abrastol a colorless or light yellow liquid is produced, while a red color is produced in presence of 0.01 g of abrastol.

**32.36****II. SANGLÉ-FERRIÈRE METHOD (17)—TENTATIVE**

Boil 200 ml of sample with 8 ml of HCl for an hour in flask fitted with reflux condenser. Abrastol is thus converted into  $\beta$ -naphthol and is detected as directed under 32.34.

**SUCROL OR DULCIN****32.37****MORPURGO METHOD (18)—TENTATIVE**

Evaporate ca 100 ml of sample, or of aqueous extract, prepared as directed under 32.1(c) and neutralized with acetic acid, to sirupy consistency after addition of ca 5 g of basic  $\text{PbCO}_3$ , and extract residue several times with 90% alcohol. Evaporate alcoholic extract to dryness, extract residue with ether, and allow the ether to evaporate spontaneously in a porcelain dish. Add 2 or 3 drops each of phenol and  $\text{H}_2\text{SO}_4$  and heat for ca 5 min. on a water bath. Cool, transfer to test tube, and overlay with  $\text{NH}_4\text{OH}$  or NaOH soln with least possible mixing. Presence of dulcin is indicated by formation of a blue color at zone of contact.

**32.38****JORISSEN METHOD (19)—TENTATIVE**

Suspend residue from ether extract obtained as directed under 32.37 in ca 5 ml of  $\text{H}_2\text{O}$ , add 2–4 ml of ca 10%  $\text{Hg}(\text{NO}_3)_2$  soln, and heat for 5–10 min. on steam bath. In presence of sucrol a violet blue color is formed. On the addition of  $\text{PbO}_2$  color changes to deep violet.

**FORMIC ACID (20)—OFFICIAL****QUANTITATIVE METHOD****32.39****REAGENTS**

(a) *Sodium acetate soln.*—Dissolve 50 g of dry Na acetate in sufficient  $\text{H}_2\text{O}$  to make 100 ml of filter.

(b) *Mercuric chloride soln.*—Dissolve 100 g of  $\text{HgCl}_2$  and 150 g of  $\text{NaCl}$  in sufficient  $\text{H}_2\text{O}$  to make 1 liter and filter.

## 32.40

## APPARATUS

The apparatus required (Fig. 54) consists of steam generator (S), 300 ml flask (A) in which sample is placed, 500 ml flask (B) containing a suspension of  $\text{BaCO}_3$ , spray trap (T), condenser, and 1 liter volumetric flask (C). The tip of tube D, leading into B, consists of a bulb containing a number of small holes to break the vapor into small bubbles.

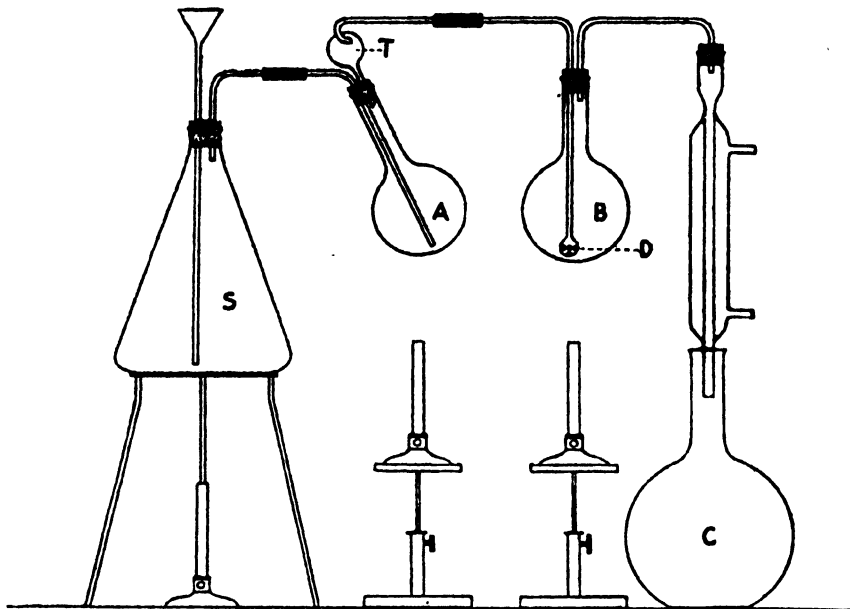


FIG. 54.—APPARATUS FOR DETERMINATION OF FORMIC ACID

## 32.41

## DETERMINATION

Use 50 ml of thin liquids like fruit juices; for heavy liquids and semi-solids like sirups and jams, use 50 g diluted with 50 ml of  $\text{H}_2\text{O}$ . Place sample in flask A, add 1 g of tartaric acid, and connect as shown in Fig. 54, the flask B having been charged previously with a suspension of 2 g of  $\text{BaCO}_3$  in 100 ml of  $\text{H}_2\text{O}$ . If much acetic acid is present, use sufficient  $\text{BaCO}_3$  so that at least 1 g remains at end of operation. Heat contents of flasks A and B to boiling and distil with steam from generator S, vapor passing first thru sample in flask A, then thru the boiling suspension of  $\text{BaCO}_3$  in B, after which it is condensed and collected in volumetric flask C. Continue distillation until 1 liter of distillate is collected, maintaining volume of liquids in flasks A and B as nearly constant as possible by heating with small Bunsen flames and avoiding charring of sample in flask A. After collecting 1 liter of distillate, disconnect apparatus and filter contents of flask B while hot, washing the  $\text{BaCO}_3$  with a little hot  $\text{H}_2\text{O}$ . Filtrate and washings should now measure ca 150 ml; if they do not, they should be boiled down to that volume. Add 10 ml of the Na acetate soln, 2 ml of

10% HCl, and 25 ml of the  $\text{HgCl}_2$  soln. Mix thoroly and immerse container in boiling water or steam bath for 2 hours. Filter thru weighed Gooch crucible and wash precipitate thoroly with cold  $\text{H}_2\text{O}$  and finally with a little alcohol. Dry in boiling water oven for 30 min., cool, weigh, and calculate weight of  $\text{HCOOH}$  present by multiplying weight of precipitate by 0.0975. If weight of  $\text{HgCl}$  obtained exceeds 1.5 g, repeat determination, using more  $\text{HgCl}_2$  or smaller quantity of sample. Conduct blank test with each new lot of reagents employed for reduction, using 150 ml of  $\text{H}_2\text{O}$ , 1 ml of 10%  $\text{BaCl}_2$  soln, 2 ml of the HCl, 10 ml of the Na acetate soln, and 25 ml of the  $\text{HgCl}_2$  soln, and heating mixture in boiling water or steam bath for 2 hours. Deduct weight of  $\text{HgCl}$  obtained in this blank test from that obtained in regular determination.

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## 33. SPICES AND OTHER CONDIMENTS

### SPICES

#### 33.1

#### PREPARATION OF SAMPLE—OFFICIAL

Grind sample to pass thru sieve having circular openings 1 mm in diam. and mix thoroly. Owing to lack of uniformity of most spices and peculiar tendency to stratify, use extreme care in weighing out portion for analysis. Stir material thoroly and weigh out 2 g sample, using spoon with capacity of ca 2 g. Dip spoonful from center of material, being careful to take ca required quantity so as to avoid adding to or taking from portion on scale pan. In determination of starch in spices by diastase method, further reduce subsample as nearly as possible to impalpable powder.

### MOISTURE

#### 33.2

#### *By Drying with Heat—Tentative*

Dry 2 g to minimum weight at 110°. From resulting loss in weight subtract quantity of volatile ether extract as determined under 33.10.

#### 33.3

#### *By Distillation with Toluene (1)—Tentative*

Clean distilling tube receiver and condenser described under 27.4, with  $\text{H}_2\text{Cr}_2\text{O}_7$ - $\text{H}_2\text{SO}_4$  mixture, rinse thoroly with  $\text{H}_2\text{O}$ , then with ca 0.5 *N* alcoholic KOH soln, and allow apparatus to drain for 10 min. Remove connecting stopper from condenser before cleaning, so that it remains dry. Place 40 g of spice in distilling flask and determine moisture as directed in 27.4.

#### 33.4

#### ASH (1)—OFFICIAL

Weigh accurately ca 2 g of sample in flat-bottomed dish, preferably of Pt. Place dish in entrance of open muffle so that sample fumes off without catching fire. Place dish in muffle kept at 550° for 30 min., break up ash with several drops of  $\text{H}_2\text{O}$ , evaporate carefully to dryness, and heat in muffle for 30 min. If previous wetting showed ash to be free from C, remove dish to desiccator containing fresh efficient desiccant ( $\text{H}_2\text{SO}_4$  or anhydrous  $\text{Mg}(\text{ClO}_4)_2$  is satisfactory), allow to cool to room temp. and weigh soon. If first wetting showed C, repeat wetting and heating until no specks of C are visible, then heat for 30 min. after disappearance of C. If C persists, leach ash with hot  $\text{H}_2\text{O}$ , filter thru quantitative filter paper, wash paper thoroly, transfer paper and contents to ashing dish, dry, and ignite in muffle at 550° until ash is white. Cool dish, add filtrate, evaporate to dryness on steam bath, and heat in muffle for 30 min. Cool, and weigh as directed previously.

*Nutmeg, mace, ginger, and cloves.*—Proceed as directed above, but heat at 600°.

*Ground mustard or mustard flour.*—Ignite as directed previously and heat for 30 min. at 550°. Leach ash with hot  $\text{H}_2\text{O}$ , filter, and wash thoroly. Transfer filter paper and contents to ashing dish, dry, and heat in muffle for 30 min. Remove dish, allow to cool, add 5–10 drops of  $\text{HNO}_3$ , evaporate to dryness, and heat in muffle 30 min. Repeat  $\text{HNO}_3$  and heating treatment until residue is white. Add filtrate, evaporate to dryness, and heat in muffle for 30 min. Cool, and weigh as directed previously.

#### 33.5

#### SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under 34.13, using ash obtained under 33.4.

**33.6 ASH INSOLUBLE IN ACID—OFFICIAL**

Boil water-insoluble residue, 33.5, or total ash, 33.4, with 25 ml of HCl (1+2.5) for 5 min., covering dish with watch-glass to prevent spattering; collect insoluble matter on Gooch crucible or on ashless filter, wash with hot H<sub>2</sub>O until washings are acid-free, ignite until C-free, cool, and weigh.

**33.7 CALCIUM OXIDE IN ASH—OFFICIAL**

Ignite 2–4 g of sample as directed under 33.4, digest with hot HCl (1+2.5), evaporate to dryness, moisten dry residue with dilute HCl, and again evaporate to dryness to render the SiO<sub>2</sub> insoluble. Treat residue with 5–10 ml of HCl, add ca 50 ml of H<sub>2</sub>O, allow to stand on water bath for a few minutes, filter, and wash insoluble residue with hot H<sub>2</sub>O. Determine CaO in combined filtrate and washings as directed under 12.12.

**33.8 NITROGEN—OFFICIAL**

Proceed as directed under 2.26. Use 1 g of sample for black or white pepper.

**33.9 NITROGEN IN NON-VOLATILE ETHER EXTRACT—OFFICIAL**

(For black and white peppers)

Extract 10 g of pepper for 20 hours in continuous extraction apparatus with absolute ether, collecting extract in weighed 250 ml flask. Evaporate ether and dry first at 100° and finally to minimum weight at 110°. Determine N in weighed extract as directed under 2.26, digesting in same flask used for extraction. Crude piperine =  $N \times 20.36$

**33.10 VOLATILE AND NON-VOLATILE ETHER EXTRACT (g)—OFFICIAL**

(Not suitable for spices high in volatile oils, such as cloves)

Extract 2 g of ground material for 20 hours in continuous extraction apparatus with anhydrous ether. Transfer ethereal soln to weighed capsule and allow to evaporate at room temp. Let stand 18 hours over H<sub>2</sub>SO<sub>4</sub> and weigh total ether extract. Heat extract gradually and then to minimum weight at 110°. The loss is volatile ether extract; the residue is non-volatile ether extract.

**33.11 ALCOHOL EXTRACT (g)—OFFICIAL**

Place 2 g of sample in 100 ml flask and fill to mark with alcohol. Stopper, shake at 30 min. intervals during 8 hours, and allow to stand 16 hours longer without shaking. Filter extract thru dry filter, evaporate 50 ml aliquot of filtrate to dryness in flat-bottomed dish on steam bath, and heat to minimum weight at 110°.

**33.12 COLD-WATER EXTRACT—TENTATIVE**

(For ginger)

Place 4 g of sample in 200 ml volumetric flask, add H<sub>2</sub>O to mark, shake at 30 min. intervals during 8 hours, and allow to stand 16 hours longer without shaking. Filter, and evaporate 50 ml aliquot of filtrate to dryness in flat-bottomed metal dish. Dry to minimum weight at 100°.

**33.13 COPPER-REDUCING SUBSTANCES BY DIRECT ACID HYDROLYSIS—OFFICIAL**

Extract 4 g of sample with 5 successive portions of 10 ml of ether on filter that will retain completely smallest starch granules. After ether has evaporated, wash with 150 ml of alcohol, 10% by volume.



To avoid clogging of filter by glutinous mass which may result from washing with  $H_2O$  or dilute alcohol, omit all preliminary washings with cassia, cassia buds, and cinnamon.

Carefully wash residue from paper into 500 ml flask with 200 ml of  $H_2O$ , using small wash bottle and gently rubbing paper with tip of finger. Hydrolyze and determine Cu reducing material, 27.33. Express result in terms of starch.

**33.14****STARCH—OFFICIAL**

Extract 4 g of finely pulverized sample with ether and 500 ml of 10% alcohol as directed under 33.13, and determine starch by diastase method, 27.35.

**33.15****CRUDE FIBER—OFFICIAL**

Proceed as directed under 27.30, and previous to weighing remove all ether extractives by successive washings of the dry fiber with ether.

**33.16****TANNIN—OFFICIAL**

(For cloves and allspice)

Extract 2 g of sample for 20 hours with anhydrous ether. Boil residue for 2 hours with 300 ml of  $H_2O$ , cool, make to 500 ml, and filter. Measure 25 ml of this infusion into 2 liter porcelain dish, add 20 ml of indigo soln, 15.32(c), and 750 ml of  $H_2O$ , and proceed as directed under 15.32. 1 ml of 0.1 *N* oxalic acid = 0.00623 g of quercitannic acid, or 0.0008 g of O absorbed.

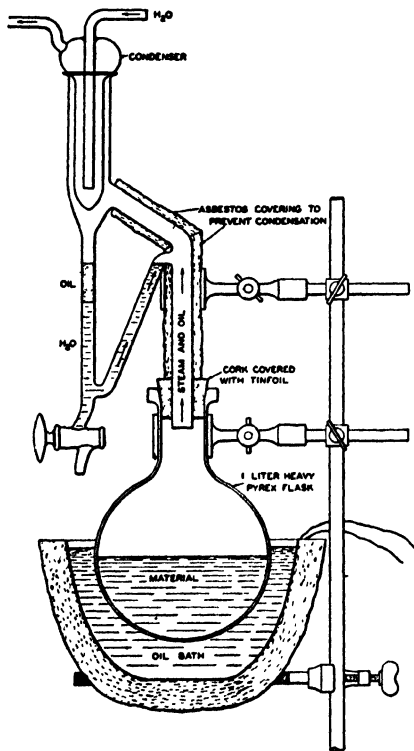


FIG. 55.—APPARATUS FOR DETERMINATION OF VOLATILE OIL

**33.17 VOLATILE OIL (4)—TENTATIVE**

Transfer weighed quantity of whole or ground material to 500–2000 ml round-bottomed, short-necked flask in amount sufficient to yield, if possible, 2 ml or more of volatile oil. Add 3–6 times as much  $H_2O$  as material and mix uniformly. Set up apparatus, Fig. 55, using appropriate volatile oil trap illustrated in Fig. 56. With oil bath (hydrogenated cottonseed oil is satisfactory) as source of heat, boil contents of flask slowly 4–8 hours, or until all volatile oil has been distilled, taking care to avoid escape of vapors around condenser. With spices (for example nutmeg) containing volatile oils lighter than  $H_2O$  and also fixed oils heavier than  $H_2O$ , discontinue distillation when fraction of oil obtained during a 1 hour period is heavier than  $H_2O$ .

In case of unsatisfactory separation of volatile oil, draw off contents of trap into small separator. After separation return  $H_2O$  to trap and transfer volatile oil to graduated cylinder. Repeat procedure if necessary.

With volatile oils heavier than  $H_2O$ , after transferring oil to graduated cylinder run off the  $H_2O$  with any remaining oil into small separator. Wash oil trap with 10 ml of ether and transfer washings to separator. Shake, and withdraw ether. Evaporate ether and drain residue into cylinder. Read quantity of volatile oil directly in cylinder and report oil in terms of ml/100 g of spice.

Allow oil to stand until perfectly clear, or dry with minimum quantity of anhydrous  $\text{Na}_2\text{SO}_4$ , and allow to settle before determining physical and chemical characteristics, 33.18–33.24.

**33.18**

### SPECIFIC GRAVITY OF VOLATILE OIL—TENTATIVE

Determine sp. gr. at 25/25° as directed under 31.3 and 31.4, using a 1 ml Sprengel tube.

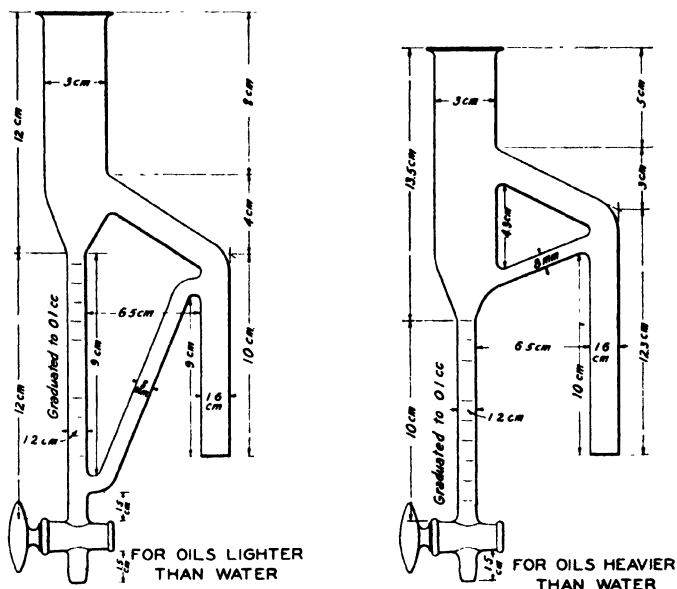


FIG. 56.--TYPES OF OIL SEPARATORY TRAPS

**33.19**

### OPTICAL ROTATION OF VOLATILE OIL—TENTATIVE

Polarize in micropolarizing tube 50 mm long and of ca 2 mm bore with white light at 25°. (Tube may be readily filled by aid of glass tube drawn out to smaller diameter.) Report in angular degrees on basis of 100 mm tube.

**33.20**

**REFRACTIVE INDEX OF VOLATILE OIL—OFFICIAL.**—See 31.8 and 31.9

**33.21**

ACID NUMBER OF VOLATILE OIL—TENTATIVE

Add 30 ml of neutral alcohol to ca 2 g of volatile oil, accurately weighed, in 200 ml Erlenmeyer flask. Titrate with 0.1 N KOH, using 1-2 drops of 1% phenolphthalein as indicator.

$$\text{Acid No.} = \frac{\text{ml } 0.1 \text{ N KOH} \times 5.61}{\text{Wt. of volatile oil}}$$

**33.22 ESTER NUMBER OF VOLATILE OIL—TENTATIVE**

To contents of flask after determination 33.21, add exactly 20 ml of 0.5 *N* KOH. Heat flask on water bath ca 2 hours, using air condenser 70–80 cm long and 5–8 mm in diam. Determine ml of 0.5 *N* KOH used in saponification (a) by titrating excess with 0.5 *N* H<sub>2</sub>SO<sub>4</sub>, using 1–2 drops of phenolphthalein as indicator.

$$\text{Ester No.} = \frac{(a) \times 28.06}{\text{Wt. of volatile oil}}.$$

**33.23 EUGENOL IN VOLATILE OIL—TENTATIVE**

Measure 2 ml of volatile oil (transfer pipet) into Babcock milk bottle, 22.26(a). Add 20 ml of 3% KOH soln, shake mixture 5 min., heat for 10 min. in boiling water bath, remove, and cool to room temp. When liquids have separated completely, add sufficient KOH soln to bring residual oil within graduated portion of neck and note volume. Calculate percentage by volume from difference of volume of sample used and residual oil.

**33.24 KETONE AND ALDEHYDE IN VOLATILE OIL (δ)—TENTATIVE**

Measure 2 ml of volatile oil (transfer pipet) into Babcock milk bottle, 22.26(a). Add 10 ml of saturated Na<sub>2</sub>SO<sub>3</sub> soln and a few drops of 1% phenolphthalein soln. Heat in bath containing boiling H<sub>2</sub>O, and shake flask repeatedly, neutralizing mixture occasionally with a few drops of saturated NaHSO<sub>3</sub> soln. If no coloration appears upon adding a few drops of phenolphthalein soln and heating for 30 min., cool to room temp. When liquids have separated completely, add sufficient Na<sub>2</sub>SO<sub>3</sub> soln to bring residual oil within graduated portion of neck and note volume. Calculate percentage by volume from difference of volume of sample used and residual oil.

**33.25 VOLATILE OIL AND RESIN IN GINGER (δ)—TENTATIVE**

Place 50 g of ground ginger in Soxhlet extractor and extract completely, using ether as solvent (ca 4 hours). Transfer extract to 300 ml flask and evaporate off ether on steam bath until solvent is no longer detected. Add 50 ml of H<sub>2</sub>O to residue and determine yield of volatile oil (using trap for oils lighter than H<sub>2</sub>O) and sp. gr., optical rotation, refractive index, acid and ester numbers as directed under 33.17–33.22.

Transfer residue in flask to separator and extract resin with ether. Transfer to tared beaker, evaporate ether on steam bath, and dry to constant weight in vacuum desiccator.

**33.26 VOLATILE OIL IN MUSTARD SEED (γ)—OFFICIAL**

Place 5 g of ground seed (No. 20 powder) in 200 ml flask, add 100 ml of H<sub>2</sub>O, stopper tightly, and macerate for 2 hours at ca 37°. Add 20 ml of alcohol and distill ca 60 ml into 100 ml volumetric flask containing 10 ml of NH<sub>4</sub>OH (1+2), taking care that end of condenser dips below surface of soln. Add 20 ml of 0.1 *N* AgNO<sub>3</sub> to distillate, set aside overnight, heat to boiling on water bath in order to agglomerate (Ag)<sub>2</sub>S, cool, make to 100 ml with H<sub>2</sub>O, and filter. Acidify 50 ml of filtrate with ca 5 ml of HNO<sub>3</sub> and titrate with 0.1 *N* NH<sub>4</sub>CNS, using 5 ml of 10% FeNH<sub>4</sub>(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O as indicator. 1 ml of 0.1 *N* AgNO<sub>3</sub> consumed = 0.004956 g of allyl isothiocyanate.

**33.27 VOLATILE OIL IN MARJORAM AND SAGE—OFFICIAL.—See 33.17**

## 33.28

## IODINE NUMBER OF PAPRIKA OIL (8)—OFFICIAL

(Qualitative test for presence of foreign oil)

Transfer 10 g of well-mixed ground sample to 200 ml glass-stoppered flask and add 100 ml of  $\text{CHCl}_3$  from pipet, rotating while adding first 50 ml. Let stand 1 hour, shake, and filter thru  $12\frac{1}{2}$  cm fluted filter paper. Pipet off successively two 10 ml portions, using same pipet. Transfer one portion to weighed crystallizing dish,  $50 \times 35$  mm, and evaporate solvent by placing dish on steam bath. Dry dish and contents at  $100^\circ$  for 1 hour, cool in air, and weigh. Use weight obtained in calculating I number. Transfer other portion to suitable glass-stoppered flask or bottle for determination of I number, 31.19, allowing 30 min. for halogen absorption. Calculate I number of  $\text{CHCl}_3$  extract. The I number of pure paprika thus obtained should be not less than 130.

## MICROSCOPIC EXAMINATION—TENTATIVE

## 33.29

## GENERAL

Adulterants of vegetable origin in spices are best detected by means of the microscope. A general knowledge of vegetable histology and the microscopic appearance of the spices and spice adulterants is essential. Some of the standard works on these subjects (9) are listed under the selected references.

## 33.30

## REAGENTS

(a) *Ammoniacal copper soln (Schweitzer reagent)*.—Add slowly a soln of  $\text{CuSO}_4$  to a soln of  $\text{NaOH}$ , leaving slight excess of  $\text{NaOH}$ ; separate by filtration the precipitate of  $\text{Cu}(\text{OH})_2$  that forms and wash it thoroly with  $\text{H}_2\text{O}$ . Dissolve wet precipitate in  $\text{NH}_4\text{OH}$  with aid of heat, cool, and filter. Prepare immediately before use and keep in dark.

(b) *Potassium hydroxide soln*.—Dissolve 5 g of  $\text{KOH}$  in  $\text{H}_2\text{O}$  and dilute to 100 ml.

(c) *Chloral hydrate soln*.—Dissolve 8 parts by weight of crystals in 5 parts of  $\text{H}_2\text{O}$ .

(d) *Acidified chloral hydrate-glycerol soln*.—Dissolve 45 g of crystals of chloral hydrate in 25 ml of  $\text{HCl}$  (1+8) and 10 ml of glycerol.

(e) *Schultze mixture*.—Mix crystallized  $\text{KClO}_3$  with  $\text{HNO}_3$  as needed.

(f) *Iodine-potassium iodide soln (iodine soln)*.—Dissolve 0.05 g of I and 0.2 g of KI in 15 ml of  $\text{H}_2\text{O}$ .

(g) *Chlorzinciodine soln*.—Dissolve 100 g of  $\text{ZnCl}_2$  in 60 ml of  $\text{H}_2\text{O}$  in glass-stoppered bottle and add 20 g of KI and 0.5 g of I crystals. Leave a few crystals of I in bottle to insure saturation, and allow soln to stand a few hours before using. (Soln will keep for months. If color developed in tissue is too deep a blue, very slight dilution of reagent is advisable.)

(h) *Ferric acetate or chloride soln*.—Use freshly prepared 1% aqueous soln.

(i) *Alkanet tincture*.—Macerate 20 g of alkanet root for several days with 100 ml of alcohol.

(j) *Safranine soln*.—Prepare saturated aqueous soln and dilute as needed.

(k) *Mayer reagent (mercuric-potassium iodide soln)*.—Dissolve 1.36 g of  $\text{HgCl}_2$  in 60 ml of  $\text{H}_2\text{O}$  and 5 g of KI in 10 ml of  $\text{H}_2\text{O}$ ; mix these two solns and dilute to 100 ml.

(l) *Millon reagent*.—See 27.13.

## 33.31

## APPARATUS

(a) *Dissecting microscope or hand lens*.—(Wide-field Greenough type microscope giving magnifications from 5–60 diameters is useful for preliminary separation.)

(b) *Compound microscope*.—Provided with objectives and oculars capable of giving range of ca 4 different magnifications varying from 75 to 500 diameters, double or triple nosepiece, and substage condenser. Eyepiece micrometer, polarizing apparatus, and mechanical stage are desirable for some special types of work.

(c) *Sieves*.—Series of standard mesh sieves varying from 10 to 100 meshes/inch and sieve having circular openings 1 mm in diameter.

(d) *Slides, cover glasses, needles, forceps, etc.*

### 33.32

#### PREPARATION OF SAMPLE

Reduce one portion to fine powder in mortar. Separate another portion into several grades of fineness by sieves of different mesh or by jarring on sheet of paper. In coarser grades fragments of suspicious nature may often be seen with naked eye or under simple microscope; these should be picked out for subsequent examination under compound microscope.

### 33.33

#### EXAMINATION

Mount small quantity of ground sample in  $H_2O$  and examine under compound microscope with both ordinary and polarized light. This gives general information as to nature of material and serves for detection and identification of starch granules and various tissues. Place small drop of the I-KI soln at edge of cover-glass, draw it into preparation by means of piece of filter paper placed at opposite edge of cover-glass, and examine again. Starch granules will be colored blue or blue-black; cellulose, yellow; and proteins, either brown or yellow.

In manner just described draw a little of the KOH soln under cover-glass and again examine. This treatment gelatinizes starch granules, dissolves proteins, saponifies fats, and in other ways clears preparation. It also imparts to tannins a reddish color. If this treatment does not clear tissues satisfactorily, treat fresh portion for short time with the acidified chloral hydrate-glycerol soln, or for some hours with the chloral hydrate soln.

Examine also crude fiber obtained in chemical analysis, as in this material stone cells and other tissues are shown distinctly.

To isolate stone cells, bast fibers, and other thick-walled cells, macerate portion of sample in Schultze mixture, using such proportions of  $KClO_3$  and  $HNO_3$  and heating for such time as will secure desired results.

To distinguish cellulose from infiltrated substances (lignin, suberin, etc.), add the freshly prepared chlorzinciodine soln to water mount. Cellulose is colored blue, and infiltrated substances are yellow.

To distinguish fats, oils, essential oils, and resins from other cell contents, treat for an hour with alkanet tincture diluted with equal volume of  $H_2O$ , which imparts to these substances a deep red color; or treat with ether, which dissolves them. Treat also with alcohol, which dissolves essential oils and resins but does not perceptibly affect fats and oils.

Test for proteins by warming cautiously on slide with a drop of freshly prepared Millon reagent. Proteins are partially decomposed, acquiring gradually a brick red color. If it is desired to study form of aleurone (protein) granules, which in some plants are quite as characteristic as starch granules, prepare mount in pure glycerol or oil.

Test for tannins and tissues impregnated with them by adding 1% Fe acetate or chloride soln. Both of these reagents give green or blue color with tannins, but Fe acetate acts more slowly and is to be preferred.

Crystals of Ca oxalate (10) are recognized by their characteristic forms and by

their behavior to polarized light. To distinguish Ca oxalate from  $\text{CaCO}_3$ , treat with acetic acid, which does not affect oxalate but dissolves carbonate with effervescence. Both are soluble in HCl.

Powdered charcoal and charred shells resist bleaching action of KOH, chloral hydrate, and Schultze mixture.

### PREPARED MUSTARD

#### 33.34

##### PREPARATION OF SAMPLE—OFFICIAL

Transfer entire contents of container to dish sufficiently large to permit thorough stirring and make whole mass homogeneous. Preserve in bottle having tightly fitted glass stopper. Stir well each time before removing portion for analysis.

#### 33.35

##### SOLIDS—OFFICIAL

Weigh 5 g of sample into flat-bottomed Pt dish; distribute evenly over bottom of dish with a little  $\text{H}_2\text{O}$ , place on steam bath until mixture appears dry, and heat in oven at  $100^\circ$  to minimum weight.

#### 33.36

##### TOTAL CHLORIDES (11)—OFFICIAL

Weigh 3–4 g of sample from weighing bottle, place in 300 ml conical flask, and add excess of standard 0.1 N  $\text{AgNO}_3$  (usually 30 ml is sufficient). Mix thoroughly and then add 15 ml of  $\text{HNO}_3$ , 5 ml at a time, rotating flask after each addition to mix contents. Add ca 50 ml of  $\text{H}_2\text{O}$  and filter into 200 ml volumetric flask. Wash filter free of  $\text{AgNO}_3$  and make filtrate to mark with  $\text{H}_2\text{O}$ . Mix thoroughly and titrate 100 ml aliquot with 0.1 N KSCN, using 2 ml of ferric alum saturated soln as indicator. Calculate chlorides as NaCl.

#### 33.37

##### ETHER EXTRACT—TENTATIVE

Weigh 10 g of sample into  $\text{SiO}_2$ , Al, or porcelain drying dish and mix with ca 30 g of sand. Heat on water bath until mixture appears dry and complete drying in water oven. Grind until all lumps are broken up, and determine ether extract as directed under 27.25.

#### 33.38

##### TOTAL NITROGEN "PROTEIN"—OFFICIAL

Determine N as directed under 2.26, using 5 g of sample. "Protein" =  $\text{N} \times 6.25$ .

#### 33.39

##### ACIDITY—OFFICIAL

Weigh 10 g of sample into 200 ml volumetric flask, dilute to mark with  $\text{H}_2\text{O}$ , shake, filter thru dry paper, and determine acidity in 100 ml by titration with 0.1 N alkali, using phenolphthalein indicator. Express result as acetic acid. 1 ml of 0.1 N alkali = 0.0060 g of acetic acid.

#### 33.40

##### COPPER-REDUCING SUBSTANCES BY DIRECT INVERSION—OFFICIAL

Proceed as directed under 27.33, except to treat directly 10 g of sample (without previous washing or extraction) with 200 ml of  $\text{H}_2\text{O}$  and 20 ml of HCl (sp. gr. 1.125), and to make up soln to 250 ml after neutralizing and before filtering and drawing off aliquot. In analyses of samples containing starch, do not have quantity of dextrose present in aliquot taken for reducing sugar determination exceed maximum permitted for that determination. Express result in terms of starch.

## STARCH (26)—TENTATIVE

## 33.41

## REAGENTS

- (a) *Calcium chloride soln.*—30 g/100 ml soln adjusted to 0.01 *N* alkalinity.  
(b) *Alcohol-NaOH soln.*—70 ml of alcohol + 30 ml of 0.1 *N* NaOH.  
(c) *Iodine-potassium iodide soln.*—2 g of I + 6 g of KI in 100 ml of H<sub>2</sub>O.

## 33.42

## DETERMINATION

Place 5 g sample of prepared mustard, or 2 g of dry mustard, in 500 ml Erlenmeyer flask and add 100 ml of the CaCl<sub>2</sub> soln from a pipet. Connect to reflux condenser, first wetting inside of condenser and stopper with H<sub>2</sub>O and draining 1 min. Heat gently to avoid initial foaming, and boil 15 min. Leaving condenser connected, cool flask in pan of H<sub>2</sub>O. Remove flask, stopper, and shake vigorously. Pour liquid into centrifuge bottle and centrifuge for several minutes. Pipet 50 ml of partly clarified middle layer into centrifuge bottle containing 150 ml of alcohol and mix well. Centrifuge at ca 1500 r.p.m. until clear. Decant liquid thru asbestos pad in Caldwell crucible, using suction. Transfer asbestos pad to same centrifuge bottle, and rinse all particles adhering to crucible into bottle with H<sub>2</sub>O. Add H<sub>2</sub>O to volume of ca 100 ml, and stopper and shake bottle vigorously until precipitate is finely dispersed. Add slight excess of the I-KI soln (2–3 ml) and 30 ml of saturated (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> soln, mix thoroly and centrifuge until clear. Filter with suction thru asbestos pad in Caldwell crucible, rinsing bottle and pad with the alcohol-NaOH soln until blue color is practically gone. Transfer asbestos pad to 500 ml Kjeldahl flask, rinsing bottle and crucible with 10 ml of HCl (sp. gr. 1.1029) followed by five 10 ml portions of H<sub>2</sub>O, carefully removing all adhering particles. Attach reflux condenser and boil 1 hour, first adding glass beads to lessen bumping. Cool, neutralize with NaOH soln (1+1), and filter into 200 ml volumetric flask; rinse flask and filter thoroly, and make to volume with H<sub>2</sub>O. Mix well, and determine dextrose as directed in 34.39 (dextrose × 0.9 = starch). (If significant, correct result for dilution due to fluid content of prepared mustard in original 100 ml of CaCl<sub>2</sub> soln.)

## 33.43

## CRUDE FIBER (18)—OFFICIAL

Weigh 10 g of sample and transfer to 8 oz. nursing bottle with 50 ml of alcohol, stopper, and shake vigorously. Add 40 ml of ether, shake, and let stand ca 5 min. with occasional shaking. Centrifuge and decant alcohol-ether mixture. Treat twice more with 40 ml portions of ether, shaking, centrifuging, and decanting as before. Rest bottle on its side for short time, without heat, to allow most of ether to evaporate. Transfer material to 500 ml Erlenmeyer flask, using 200 ml of boiling H<sub>2</sub>SO<sub>4</sub>, 27.28(a), and proceed as directed under 27.30, but in addition wash fiber with successive portions of ether previous to drying and weighing.

If preferred, treat sample with alcohol and ether in small beaker, transfer to hardened 11 cm filter paper, wash several times with ether, and transfer to 500 ml Erlenmeyer flask with 200 ml of boiling H<sub>2</sub>SO<sub>4</sub>.

## 33.44

## COLORING MATTER—TENTATIVE.—See Chap. 21

## 33.45

## PRESERVATIVES—OFFICIAL.—See Chap. 32

## MAYONNAISE AND SALAD DRESSING (19)

## 33.46

## PREPARATION OF SAMPLE—TENTATIVE

Before removing any portion of sample for analysis, transfer to suitable container, such as glass fruit jar of larger capacity than volume of sample, and mix until

homogeneous with spatula (2–3 min. should be sufficient). Repeat mixing before each subsequent portion is removed for analysis if sample has stood for any appreciable length of time. For the various determinations, take ca quantity directed and weigh. (Light 100 ml flask fitted with straight glass tube and over-sized rubber bulb makes suitable weighing bottle.)

**33.47****TOTAL SOLIDS—OFFICIAL**

Use 2 g sample and proceed as directed in 23.3.

**33.48****REDUCING SUGARS BEFORE INVERSION—OFFICIAL, FIRST ACTION**

Weigh 20 g of sample into wide-mouthed, 4 oz. bottle and extract oil by adding ca 80 ml of petroleum benzine, shaking, and centrifuging. Draw off as much as possible of petroleum benzine soln (conveniently done by using suction and short-stemmed pipet), and repeat treatment with petroleum benzine until all oil has been removed (indicated by absence of color in solvent—usually four extractions are required). Reserve ether soln for identification of oil. Remove petroleum benzine from residue with current of air and transfer residue with  $H_2O$  to 100 ml volumetric flask. Add 5–10 ml of fresh soln of  $HPO_3$  (remove any white coating on  $HPO_3$  by rinsing with  $H_2O$ , dissolve 5 g of transparent lumps or sticks in cold  $H_2O$  and dilute to 100 ml), mix thoroly, dilute to volume, and filter. Transfer 80 ml of filtrate, or as large an aliquot as possible, to 100 ml flask; neutralize with NaOH soln (1+1), using phenolphthalein indicator; cool, dilute to mark, and determine reducing sugars on aliquot as directed under 34.39. Calculate to invert sugar.

With dressings, particularly those containing starch, that cannot be clarified by above method, remove oil as directed under 22.25, using 1 ml of  $NH_4OH$  and 5 ml of alcohol for each gram of sample; transfer residue to 250 ml flask with alcohol, 50% by volume, and proceed as directed under 27.31 and 34.39.

**33.49****REDUCING SUGARS AFTER INVERSION—OFFICIAL, FIRST ACTION**

Invert aliquot of the soln, 33.48, as directed under 34.24(b) or (c), nearly neutralize with NaOH soln (1+1), and determine reducing sugars in inverted soln as directed under 34.39. Calculate to invert sugar from 44.11.

**33.50****SUCROSE—OFFICIAL, FIRST ACTION**

Subtract percentage of invert sugar obtained before inversion, 33.48, from that obtained after inversion, 33.49, and multiply difference by 0.95.

**33.51****TOTAL ACIDITY—OFFICIAL**

Weigh ca 15 g of sample into 500 ml Erlenmeyer flask, dilute to ca 200 ml, and shake until all lumps of dressing are thoroly broken up. Titrate with 0.1 *N* NaOH, using neutral phenolphthalein, and calculate as acetic acid. In order to recognize the end point, have duplicate sample at hand so that, by comparison, first change of color may be noted.

**33.52****TOTAL NITROGEN—OFFICIAL**

Weigh ca 15 g of sample into 500 ml Kjeldahl flask and place on steam bath until egg is thoroly cooked and oil separates readily. Cool, and add ca 50 ml of petroleum benzine; mix, and pour off benzine soln thru small filter. Repeat benzine treatment twice, rinsing out as much oil as possible. Wash filter with petroleum benzine and add filter paper to sample in flask. Determine N, using 35 ml of  $H_2SO_4$  for digestion as directed in 2.26.



**33.53 TOTAL PHOSPHORIC ACID (P<sub>2</sub>O<sub>5</sub>)—OFFICIAL**

Use 10 g sample and proceed as directed in 23.19 and 23.20, except to use Pt dish in place of beaker and to burn off oil before ashing in muffle.

**33.54 TOTAL FAT—TENTATIVE**

Use 1 g sample and proceed as directed under 23.8 and 23.9.

**33.55 CALCULATION OF COMPOSITION—TENTATIVE**

When  $P = \% \text{ total P}_2\text{O}_5$  and  $N = \% \text{ total N}$ , then  $\% \text{ yolk} = 75.69 P - 1.802 N$ ;  
 $\% \text{ white} = 60.80 N - 114.59 P$ ;  $\% \text{ total egg} = \% \text{ yolk} + \% \text{ white}$ ;  $\% \text{ white in egg}$

$\% \text{ white}$   
 $\text{component} = \frac{\% \text{ white}}{\% \text{ total egg}} \times 100$ ; vegetable oil = total fat — (yolk  $\times 0.3188$ ); vinegar

(4% acid strength) = total acidity as acetic  $\times 25$ ; minor constituents (sugar, salt, spices, stabilizers) = total solids — (yolk  $\times 0.5047$ ) — white  $\times 0.1221$ ) — vegetable oil; and added water = 100% — total egg — vegetable oil — vinegar — minor constituents.

**33.56 IDENTIFICATION OF OIL—OFFICIAL**

Proceed as directed under Chap. 31, using oil obtained by evaporating petroleum benzine extracts from determination of reducing sugars, 33.48.

**33.57 GUMS IN MAYONNAISE AND FRENCH DRESSING (14)—TENTATIVE**

Transfer 100 g into 250 ml beaker, add 35–40 ml of hot H<sub>2</sub>O, and mix thoroly. Heat to 65–70° in water bath, add 10 ml of 50% trichloroacetic acid soln, and maintain at 65–70° until emulsion shows signs of breaking (in no case over 10 min.). Transfer mixture to 8 oz. nursing bottle, insert pipet guard (15), and centrifuge 15–20 min. at ca 1200 r.p.m. (This should separate mixture into lower aqueous layer and upper oily layer, with layer of curd between. If separation does not occur, add 30–40 ml of toluene, mix, and repeat centrifuging.) By means of pipet inserted thru pipet guard remove as much of aqueous layer as possible and filter it into 600 ml beaker. Add 5 volumes of alcohol and allow mixture to stand overnight to precipitate gums.

Decant or pipet off sufficient alcohol to leave not over 225 ml, transfer contents of beaker to 8 oz. nursing bottle, centrifuge until gum settles to bottom, and decant supernatant alcohol as completely as possible. Dissolve residue in not over 1.5 oz. of hot H<sub>2</sub>O, add 1 or 2 ml of acetic acid, and reprecipitate by adding alcohol to 8 oz. mark on nursing bottle. Let stand overnight, or until precipitate becomes flocculent, centrifuge at 1200 r.p.m., and decant alcohol. Presence of significant amount of gum will be denoted by heavy flocculent precipitate at this point. Slight precipitate should not be considered a positive test for gums, as spices present in most mayonnaises and French dressings will usually give such a precipitate. Confirm presence of gums by following procedure:

Add 35 ml of hot H<sub>2</sub>O to precipitate in nursing bottle, transfer to small beaker, add 5 ml of HCl, and boil gently 2 min. to hydrolyze gums to sugars. This soln may now be used for various qualitative tests for monosaccharide sugars, as follows:

(a) *Copper reduction test.*—Transfer 1 ml of hydrolyzed gum soln to test tube neutralize with ca 2 N NaOH, using litmus paper as indicator, remove litmus paper add 5 ml of Benedict qualitative sugar soln, 22.138(a), and boil vigorously 1–2 min. Allow to cool spontaneously. A voluminous precipitate, which may be green, yellow, or red, indicates reducing sugars.

(b) *Molisch test*.—Transfer 5 ml of hydrolyzed gum soln to test tube, and add 2 drops of 15% soln of  $\alpha$ -naphthol in alcohol. Incline tube and slowly pour down inner side 3–5 ml of  $H_2SO_4$  so that two layers will not mix. Reddish-violet zone at point of contact indicates carbohydrates. (5% soln of thymol in alcohol may be substituted for  $\alpha$ -naphthol.)

### VINEGARS (16)

(Unless otherwise directed, express results as g/100 ml)

#### 33.58

##### ORGANOLEPTIC EXAMINATION—OFFICIAL

Note appearance, color, odor, and taste. Neutralize portion of sample with NaOH soln and note odor and taste. Extract neutralized vinegar with ether, evaporate ether extract, and note odor and taste of residue. (Spices and pungent materials are indicated by characteristic odors and tastes.) Evaporate portion of sample on water bath. Odor of material as last of volatile matter evaporates and appearance and taste of residue give information as to source and character of vinegar.

#### 33.59

##### PREPARATION OF SAMPLE—OFFICIAL

Mix thoroly and filter thru rapidly acting filter paper before proceeding with analysis.

#### 33.60

##### SOLIDS—OFFICIAL

Measure 10 ml of sample into weighed, flat-bottomed Pt dish having bottom diameter of 50 mm, evaporate on boiling water bath for 30 min., and dry for exactly 2.5 hours in water oven at temp. of boiling  $H_2O$ . Cool in desiccator and weigh. To obtain concordant results, it is necessary to use a dish of size and shape stated and to dry for exactly time specified.

#### 33.61

##### ASH—OFFICIAL

Measure 25 ml of vinegar into weighed Pt dish, evaporate to dryness on water or steam bath, and heat in muffle at 500–550° for 30 min. Break up charred mass in Pt dish, add hot  $H_2O$ , filter thru ashless filter, and wash *thoroly* with  $H_2O$ . Return filter and contents to dish, dry, and heat at ca 525° for 30 min., or until all C is burned off. Add filtrate, evaporate to dryness, and heat at ca 525° for 15 min. Cool in desiccator and weigh (Weight A). Reheat in muffle at ca 525° for 5 min., and cool for not more than 1 hour in desiccator containing efficient desiccant. Put no more than 2 dishes, preferably only 1, in desiccator at one time. Place Weight A on balance pan before removing dish from desiccator, and weigh rapidly to milligrams. Calculate total ash from last weight.

#### 33.62

##### SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Treat ash, 33.61, as directed under 34.13.

#### 33.63

##### ALKALINITY OF SOLUBLE ASH—OFFICIAL

Proceed as directed under 34.14, using soluble ash obtained under 33.62. Express result as number of ml of *N* acid required to neutralize soluble ash from 100 ml of vinegar. If relationship of ash to alkalinity of soluble ash is abnormal, study composition of ash, especially as to content of chlorides, sulfates, phosphates, and alkalis (17).

**33.64 SOLUBLE PHOSPHORIC ACID (18)—TENTATIVE**

Proceed as directed under 2.9 or 2.12, or 26.50, using soln obtained under 33.63. If either volumetric or colorimetric method is used, standardize with sample of known phosphate content. Express results as mg of  $P_2O_5$ /100 ml of vinegar.

**33.65 INSOLUBLE PHOSPHORIC ACID (18)—TENTATIVE**

Dissolve water-insoluble ash, 33.62, in ca 50 ml of boiling  $HNO_3$  (1+8) (use 25 ml of  $H_2SO_4$  (1+9) for colorimetric method) and proceed as directed under 2.9 or 2.12, or 26.50. If either volumetric or colorimetric method is used, standardize with sample of known phosphate content. Express result as mg of  $P_2O_5$ /100 ml of vinegar.

**33.66 TOTAL PHOSPHORIC ACID (18)—OFFICIAL**

Dissolve ash, 33.61, or both soluble and insoluble ash, 33.62, in ca 50 ml of boiling  $HNO_3$  (1+8) (use 25 ml of  $H_2SO_4$  (1+9) for colorimetric method) and proceed as directed under 2.9 or 2.12, or 26.50. If either volumetric or colorimetric method is used, standardize with sample of known phosphate content. Express result as mg of  $P_2O_5$ /100 ml of vinegar. If desired, digest vinegar as directed in 26.49, instead of using ash from 33.61.

**33.67 TOTAL ACIDS—OFFICIAL**

Dilute 10 ml of sample with recently boiled and cooled  $H_2O$  until it appears slightly colored and titrate with 0.5 *N* alkali, using phenolphthalein indicator. 1 ml of 0.5 *N* alkali = 0.0300 g of acetic acid.

**33.68 NON-VOLATILE ACIDS—OFFICIAL**

Measure 10 ml of vinegar into 200 ml porcelain casserole, evaporate just to dryness, add 5–10 ml of  $H_2O$ , and again evaporate; repeat until at least 5 evaporations have been made. Add ca 200 ml of recently boiled and cooled  $H_2O$  and titrate with 0.1 *N* alkali, using phenolphthalein indicator. 1 ml of 0.1 *N* alkali = 0.00600 g of acetic acid.

**33.69 VOLATILE ACIDS—OFFICIAL**

Subtract quantity of non-volatile acids, 33.68, from quantity of total acids, 33.67.

**33.70 TOTAL REDUCING SUBSTANCES BEFORE INVERSION—OFFICIAL**

Measure 25 ml of sample into 50 ml volumetric flask and add enough  $NaOH$  soln (1+1) nearly to neutralize acid. Cool, dilute to mark with  $H_2O$ , and determine reducing substances in 20 ml of the soln as directed under 34.39. If quantity of reducing substances is very small, use 40 ml. Calculate result as invert sugar (for malt vinegar as dextrose).

**33.71 TOTAL REDUCING SUBSTANCES AFTER INVERSION—OFFICIAL**

Invert 25 ml of sample in 50 ml volumetric flask with 5 ml of  $HCl$ , as directed under 34.24(b) or (c). Nearly neutralize with  $NaOH$  soln (1+1) and determine reducing substances as directed under 34.39.

**33.72 NON-VOLATILE REDUCING SUBSTANCES (SUGAR)—OFFICIAL**

(Useful in calculating non-sugar solids)

Evaporate 50 ml of sample on steam or water bath to sirupy consistency, add 10 ml of  $H_2O$ , and evaporate again. Repeat with 10 ml of  $H_2O$ . Transfer residue to

100 ml volumetric flask with ca 50 ml of warm  $\text{H}_2\text{O}$ . Cool; invert with 10 ml of  $\text{HCl}$  as directed under 34.24(b) or (c); nearly neutralize with  $\text{NaOH}$  soln (1+1); cool, dilute to mark with  $\text{H}_2\text{O}$ , and determine reducing substances in 20 ml or 40 ml, depending on quantity present, as directed under 34.39. Calculate result as invert sugar (for malt vinegar as dextrose). If results for total reducing substances before and after inversion show absence of sucrose, inversion may be omitted.

### 33.73 VOLATILE REDUCING SUBSTANCES (19)—OFFICIAL

When sucrose is absent, subtract quantity of non-volatile reducing substances, 33.72, from mean of total reducing substances before inversion, 33.70, and after inversion, 33.71. When sucrose is present, subtract quantity of non-volatile reducing substances, 33.72, from quantity of total reducing substances after inversion, 33.71.

### 33.74 ALCOHOL—OFFICIAL

Measure 100 ml of sample into round-bottomed distillation flask. Make faintly alkaline with  $\text{NaOH}$  soln (1+1), distil almost 50 ml, dilute to 50 ml at temp. of sample, and determine sp. gr. at 20/20° by means of pycnometer, 16.4. Obtain from 44.23 percentage by volume. Undue foaming may be obviated by adding small piece of paraffin, free from volatile constituents.

### GLYCEROL (20)—OFFICIAL

#### 33.75

#### REAGENTS

(a) *Strong potassium dichromate soln.*—Dissolve 74.55 g of dry, recrystallized  $\text{K}_2\text{Cr}_2\text{O}_7$  in  $\text{H}_2\text{O}$ ; add 150 ml of  $\text{H}_2\text{SO}_4$ ; cool, and dilute with  $\text{H}_2\text{O}$  to 1 liter at 20°. 1 ml of this soln = 0.01 g of glycerol. Owing to high coefficient of expansion of this strong soln it is necessary to make all volumetric measurements of soln at same temp. as that at which it was diluted to volume.

(b) *Dilute potassium dichromate soln.*—Measure 25 ml of the strong  $\text{K}_2\text{Cr}_2\text{O}_7$  soln at 20° into 500 ml volumetric flask and dilute to mark with  $\text{H}_2\text{O}$  at room temp. 20 ml of this soln = 1 ml of (a).

(c) *Ferrous ammonium sulfate soln.*—Dissolve 30 g of  $\text{FeSO}_4 \cdot (\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ , add 50 ml of  $\text{H}_2\text{SO}_4$ , cool, and dilute with  $\text{H}_2\text{O}$  to 1 liter at room temp. 1 ml of this soln = approximately 1 ml of (b). As its value changes slightly from day to day, it must be standardized against (b) whenever used.

(d) *Diphenylamine indicator.*—Dissolve 1 g of diphenylamine in 100 ml of  $\text{H}_2\text{SO}_4$ .

(e) *Retarder.*—Dilute 150 ml of  $\text{H}_3\text{PO}_4$  with 600 ml of  $\text{H}_2\text{O}$ , and add 250 ml of  $\text{H}_2\text{SO}_4$ .

(f) *Milk of lime.*—Introduce 150 g of  $\text{CaO}$ , selected from clean hard lumps, prepared preferably from marble, into large porcelain or iron dish; slake with  $\text{H}_2\text{O}$ , cool, and add sufficient  $\text{H}_2\text{O}$  to make 1 liter.

(g) *Silver carbonate.*—Dissolve 0.1 g of  $\text{Ag}_2\text{SO}_4$  in ca 50 ml of  $\text{H}_2\text{O}$ , add an excess of  $\text{Na}_2\text{CO}_3$  soln, allow precipitate to settle, and wash with  $\text{H}_2\text{O}$  several times by decantation until washings are practically neutral. This reagent must be freshly prepared immediately before use.

### 33.76

#### DETERMINATION

Make evaporations on water bath maintained at temp. of 85–90°. Area of dish exposed to bath should not be greater in circumference than that covered by liquid inside.

Evaporate 100 ml of vinegar to 5 ml, add 20 ml of  $H_2O$ , and again evaporate to 5 ml to expel acetic acid. Treat residue with ca 5 g of 40-mesh sand and 15 ml of the milk of lime and evaporate almost to dryness, with frequent stirring, avoiding formation of dry crust or evaporation to complete dryness. Treat moist residue with 5 ml of  $H_2O$ ; rub to homogeneous paste; add slowly 45 ml of absolute alcohol, washing down sides of dish to remove adhering paste; and stir thoroly. Heat mixture on water bath, with constant stirring, to incipient boiling; transfer to suitable vessel and centrifuge. Decant clear liquid into porcelain dish and wash residue with several small portions of hot alcohol, 90% by volume, by aid of centrifuge. (If centrifuge is not available, decant liquid thru folded filter into porcelain dish. Wash residue repeatedly with small portions of hot 90% alcohol, twice by decantation, and then by transferring all material to filter. Continue washing until filtrate amounts to 150 ml.) Evaporate to sirupy consistency, add 10 ml of absolute alcohol to dissolve residue, and transfer to 50 ml glass-stoppered cylinder, washing dish with successive small portions of absolute alcohol until volume of soln is 20 ml. Add 3 portions of 10 ml each of anhydrous ether, shaking thoroly after each addition. Let stand until clear, pour off thru filter, and wash cylinder and filter with mixture of 2 volumes of absolute alcohol and 3 of anhydrous ether. If heavy precipitate has formed in cylinder, centrifuge at low speed, decant clear liquid, and wash 3 times with 20 ml portions of the alcohol-ether mixture, shaking mixture thoroly each time and separating precipitate by means of centrifuge. Wash paper with the alcohol-ether mixture and evaporate filtrate and washings on water bath to ca 5 ml; add 20 ml of  $H_2O$ , and again evaporate to 5 ml; again add 20 ml of  $H_2O$  and evaporate to 5 ml; finally add 10 ml of  $H_2O$  and evaporate to 5 ml.

These evaporations are necessary to remove all ether and alcohol, and when conducted at 85–90° they result in no loss of glycerol if concentration of latter is less than 50%.

Transfer residue with hot  $H_2O$  to 50 ml volumetric flask, cool, add  $Ag_2CO_3$  prepared from 0.1 g of  $Ag_2SO_4$ , shake, and allow to stand 10 min. Add 0.5 ml of basic Pb acetate soln, 34.19(a); shake occasionally, and allow to stand 10 min. Make to mark, shake well, and filter, rejecting first portion of filtrate. Pipet 25 ml of clear filtrate into 250 ml volumetric flask.

Add 1 ml of  $H_2SO_4$  to precipitate excess of Pb and then 30 ml of Reagent (a). Add carefully 24 ml of  $H_2SO_4$ , rotating flask gently to mix contents and avoid violent ebullition, and then place in *boiling* water bath for exactly 20 min. Remove flask from bath, dilute, cool, and make to mark at room temp. Quantity of strong dichromate soln used must be sufficient to leave excess of ca 12.5 ml at end of oxidation. (Quantity given above, 30 ml, is sufficient for ordinary vinegar containing ca 0.35 g or less of glycerol/100 ml.)

Standardize the  $Fe(NH_4)_2(SO_4)_2$  soln by pipetting 20 ml into 250 ml beaker, adding 20 ml of the retarder, 4 drops of the indicator, and ca 100 ml of  $H_2O$ . Titrate with the dilute  $K_2Cr_2O_7$  soln until liquid assumes dark green color, then add  $K_2Cr_2O_7$  slowly dropwise, stirring continuously, until color changes from blue gray to deep violet. Designate ml of dilute  $K_2Cr_2O_7$  soln used as (a). In place of the dilute  $K_2Cr_2O_7$  soln, substitute buret containing the oxidized glycerol and excess strong  $K_2Cr_2O_7$  soln, and titrate 20 ml of the  $Fe(NH_4)_2(SO_4)_2$  soln as before, designating ml used as (b).

From figures obtained calculate glycerol by following formula:

$$G = \left( D - \frac{250(a)}{20(b)} \right) 0.02, \text{ in which}$$

$G$  = g of glycerol/100 ml of vinegar, and  $D$  = ml of strong  $K_2Cr_2O_7$  soln used to oxidize glycerol.

## 33.77

## COLOR—OFFICIAL

Determine depth of color in Lovibond tintometer by good reflected daylight, using  $\frac{1}{2}$  or 1" cell and the brewers' scale. Report result in terms of  $\frac{1}{2}$ " cell and so state.

## CAMEL (#1)—TENTATIVE

## 33.78

## REAGENTS

(a) *Stock soln.*—Dissolve 9.8 g of tannic acid in ca 300 ml of  $H_2O$ , add 4 ml of  $H_2SO_4$ , and dilute to 490 ml. Allow to stand overnight before use. (This soln keeps for a considerable time.)

(b) *Modified Lichthardt soln.*—Add 4 ml of 40% formaldehyde soln to 96 ml of the stock soln. Mix well and filter. Prepare fresh reagent daily.

## 33.79

## QUALITATIVE TEST

Mix 5 ml of sample and 5 ml of (b) in test tube and immerse in bath of boiling  $H_2O$  for 4 min. Remove from bath, cover, and allow to stand overnight. (Brown precipitate settled to bottom of tube indicates positive test for caramel. Gray precipitate should be disregarded.)

## 33.80

## OTHER COLORING MATTERS—TENTATIVE.—See Chap. 21

## 33.81

## POLARIZATION (#2)—TENTATIVE

Whenever possible, polarize in 200 mm tube without decolorizing. Report results on basis of 200 mm tube in degrees Ventzke. When necessary, decolorize as follows:

(a) To 50 ml of sample add measured quantity of saturated neutral Pb acetate soln, avoiding excess of Pb; filter, remove Pb with powdered anhydrous K oxalate, and filter. Polarize and correct for dilution with Pb acetate soln.

(b) To 50 ml of sample add decolorizing C, avoiding excessive amount or length of treatment. Filter thru double paper and polarize.

## 33.82

## SULFATES—OFFICIAL

To 100 ml of sample, add 2 ml of ca normal HCl; heat to boiling; add 10 ml of hot  $BaCl_2$  soln (1 g/100 ml), dropwise, and continue boiling 5 min., keeping volume ca constant by adding hot  $H_2O$  as required. Allow mixture to stand until supernatant liquid is clear. (Overnight is convenient, but this time should not be exceeded.) Filter on ashless paper or weighed Munroe crucible (#3). Wash free from chlorides with hot  $H_2O$ , dry, ignite at low red heat, cool, and weigh. Express result as mg of  $SO_3$ /100 ml of vinegar.

## TARTARIC ACID AND TARTRATES

## 33.83

## Qualitative Test—Official

Evaporate 50 ml of sample in porcelain dish to volume of ca 10 ml, filter, add 1 ml of 25%  $CaCl_2$  soln and 2 ml of 50%  $NH_4$  acetate soln, and allow to stand overnight. In presence of tartaric acid, deposit of Ca tartrate is formed, crystals of which may be identified under microscope by their characteristic form.

## 33.84

## Quantitative Determination—Official

Evaporate 200 ml of sample to sirupy consistency to remove excess of acetic acid, dilute to original volume with  $H_2O$  in volumetric flask, determine acidity as directed under 33.67, and determine total tartaric acid in 100 ml aliquot as directed under 15.28, using 20 ml of alcohol in precipitation instead of 15 ml.

## FREE MINERAL ACIDS

33.85

*Logwood Method (24)—Tentative*

Prepare extract of logwood as follows: Pour 100 ml of boiling  $H_2O$  upon 2 g of fresh logwood chips, allow infusion to stand for a few hours, and filter. Place 3–4 drops of the liquid in depression of porcelain spot plate and dry on water bath. Add to residue 1–2 drops of sample and allow to stand 2–3 min. (do not allow complete evaporation). Yellow tint remains if free mineral acids are absent, red tint if they are present. Test is improved if sample is first decolorized by treatment with Nuchar or similar vegetable carbon and filtration.

33.86

*Methyl Violet Method—Tentative*

Add 5–10 ml of  $H_2O$  to 5 ml of vinegar, and after mixing well add 4 or 5 drops of methyl violet soln [1 part of methyl violet (C. I. 680; S. & J. 451) in 10,000 parts of  $H_2O$ ]. Blue or green coloration indicates presence of free mineral acid.

33.87

*Quantitative Method—Tentative*

To measured quantity of sample add measured excess of standard alkali, evaporate to dryness, incinerate at ca  $525^\circ$ , and titrate ash with standard acid, using methyl orange indicator. Difference between number of ml of alkali first added and number of ml of acid of same normality needed to titrate ash = ml of free mineral acid present.

33.88

## METALS—TENTATIVE.—See Chap. 29

33.89

## DEXTRIN (QUALITATIVE TEST)—TENTATIVE

Evaporate 100 ml of the vinegar to volume of ca 15 ml. Add slowly and with constant stirring 200 ml of alcohol and allow to stand overnight. Separate precipitate, preferably by centrifuging, and wash with 80% alcohol. Dissolve in minimum quantity of  $H_2O$  and determine optical rotation, 26.56. (Distinct optical rotation indicates dextrin.) Treat soln with several drops of I soln of ca same color intensity. Formation of reddish brown color indicates dextrin.

33.90

## PRESERVATIVES—OFFICIAL.—See Chap. 32

## PERMANGANATE OXIDATION NUMBER (26)—TENTATIVE

(For differentiating between vinegar and commercial acetic acid)

33.91

## REAGENTS

(a) *Acetic acid soln.*—To 4 g of acetic acid add 100 ml of  $H_2O$ . (This soln should have negligible permanganate oxidation number.)

(b) *Sulfuric acid soln.*—(1 + 1).

(c) *Potassium iodide soln.*—Dissolve 30 g of KI in 100 ml of  $H_2O$  and filter. Do not use unless colorless.

33.92

## DETERMINATION

Adjust vinegar to 4 g/100 ml acidity as acetic acid. Steam distil 50 ml of adjusted vinegar, maintaining volume so as to have residue of 45 ml for 50 ml of distillate. (All glass apparatus is preferable; if not available, cork or rubber stoppers should be covered with Sn or Al foil.) Keep distillate and reagents at  $25^\circ$ . Transfer the 50 ml

distillate to 250 ml glass-stoppered Erlenmeyer flask. Add 10 ml of the  $\text{H}_2\text{SO}_4$  soln and 25 ml of normal  $\text{KMnO}_4$  soln. Hold at  $25^\circ$ , preferably in  $\text{H}_2\text{O}$  bath, for exactly 1 hour. Then immediately add 20 ml of the  $\text{KI}$  soln and mix well. Titrate the freed  $\text{I}$  with  $0.5\text{ }N\text{ } \text{Na}_2\text{S}_2\text{O}_3$ . Run blank on the acetic acid soln and subtract normal  $\text{KMnO}_4$  used from the 25 ml before subtraction of the ml of the  $0.5\text{ }N\text{ } \text{Na}_2\text{S}_2\text{O}_3$ .

To obtain the permanganate oxidation number divide by 2 the  $\text{Na}_2\text{S}_2\text{O}_3$  soln used and subtract this from 25. If the permanganate oxidation number is more than 15, repeat, taking half the original quantity of vinegar. Repeat this reduction by half until the ml of normal  $\text{KMnO}_4$  soln used is less than 15. Calculate permanganate oxidation number to basis of 50 ml sample.

If desirable run the permanganate oxidation number on 50 ml of the adjusted vinegar without distillation and also on the undistilled residue made up to 50 ml with  $\text{H}_2\text{O}$ .

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## 34. SUGARS AND SUGAR PRODUCTS

### SUGARS, SIRUPS, AND MOLASSES

#### 34.1

##### PREPARATION OF SAMPLE—OFFICIAL

(a) *Solids (sugars, etc.)*.—Grind, if necessary, and mix thoroly to secure uniform samples. In case of raw sugars mix thoroly and in shortest possible time on glass plate with spatula, reducing lumps when present with glass or iron rolling pin; or mix thoroly and in shortest possible time in large, clean, dry mortar, using pestle to reduce lumps when present.

(b) *Semi-solids (massecuites, etc.)*.—Weigh 50 g of sample, dissolve crystals of sugar in minimum amount of  $H_2O$ , wash into 250 ml volumetric flask, fill to mark, and mix thoroly; or weigh 50 g of sample and dilute with  $H_2O$  to 100 g. If insoluble material remains, mix uniformly by shaking before taking aliquots or weighed portions for various determinations.

(c) *Liquids (molasses, sirups, etc.)*.—Mix materials thoroly. If crystals of sugar are present, dissolve them either by heating gently (avoiding loss of  $H_2O$  by evaporation) or by weighing whole mass, then adding  $H_2O$ , heating until completely dissolved and after cooling, reweighing. Calculate all results to weight of original substance.

### MOISTURE

#### 34.2

##### *Direct Drying—Tentative*

Dry 2–5 g of prepared sample, 34.1(a), in flat dish (Ni, Pt, or Al) at  $100^\circ$  for 10 hours; cool in desiccator and weigh. Dry again for 1 hour or until change in weight is not more than 2 mg. In case of sugars of large grain, heat at  $105$ – $110^\circ$  to expel last traces of occluded  $H_2O$  (1). Report loss in weight as moisture.

#### 34.3

##### *Vacuum Drying Method—Official*

(Applicable to both cane and beet raw and refined sugars)

Dry 2–5 g of prepared sample, 34.1(a), in a flat dish (Ni, Pt, or Al) with tight-fitting cover, at temp. not exceeding  $70^\circ$  (preferably  $60^\circ$ ), under pressure not exceeding 50 mm of Hg, for 2 hours. Remove dish from oven, put cover in place, cool in desiccator, and weigh. Re-dry 1 hour and repeat process until change in weight between successive weighings at 1 hour intervals is not more than 2 mg. (Oven should be bled with current of dry air during drying to insure removal of water vapors.)

#### 34.4

##### *Drying upon Pumice Stone—Official*

(Applicable to massecuites, molasses, and other liquid and semiliquid products)

Prepare pumice stone of 2 grades of fineness, one of which will pass thru 1 mm sieve, the other thru 6 mm but not 1 mm sieve. Digest each with  $H_2SO_4$  (1+4) for 8 hours on steam bath. Wash free from acid and heat to dull redness. Make determination in flat metallic dish 60 mm in diam. Place layer of the fine pumice stone, 3 mm in thickness, on bottom of dish, then layer of the coarse pumice stone, 6–10 mm in thickness; dry, and weigh. Dilute sample with weighed portion of  $H_2O$  so that diluted material will contain 20–30% of solid matter. Weigh into dish, prepared as described, quantity of diluted sample to yield ca 1 g of dry mat-

ter. If this weighing cannot be made rapidly, use weighing bottle provided with cork thru which pipet passes. Dry at 70° under pressure not to exceed 50 mm of Hg, making trial weighings at intervals of 2 hours toward end of drying period until change in weight does not exceed 2 mg. Report loss in weight as moisture. For substances containing little or no levulose or other readily decomposable substance, drying may be made in oven at 100°.

## 34.5

*Drying upon Quartz Sand (2)—Official*

(Applicable to massecuites, molasses, and other liquid and semiliquid products)

Digest pure quartz sand that will pass 40-mesh but not 60-mesh sieve with HCl, wash free from acid, dry, and ignite. Preserve in stoppered bottle. Place 25–30 g of prepared sand and short stirring rod in dish ca 55 mm in diam. and 40 mm in depth, fitted with cover. Dry thoroly, cover dish, cool in desiccator, and weigh immediately. Add sufficient diluted sample of known weight to yield ca 1 g of dry matter and mix thoroly with the sand. Heat on steam bath 15–20 min., stirring at intervals of 2–3 min., or until mass becomes too stiff to manipulate readily. Dry at 70° under pressure not exceeding 50 mm of Hg, making trial weighings at 2 hour intervals toward end of drying period (ca 18 hours) until change in weight does not exceed 2 mg.

For materials containing no levulose or other readily decomposable substance dry at atmospheric pressure in oven at 100°, heat 8–10 hours, cool in desiccator, and weigh, repeating heating and weighing until loss in 1 hour does not exceed 2 mg. Report loss in weight as moisture.

As dry sand, as well as dried sample, will absorb appreciable quantity of moisture on standing over most desiccating agents, make all weighings as quickly as possible after cooling in desiccator.

## SOLIDS

## 34.6

*By Means of Spindle—Official*

(Not accurate when applied to low-grade sugar products, molasses, and other materials containing large quantities of non-sugar solids, but extensively used for approximate results)

Density of juices, sirups, etc., is conveniently determined by means of Brix or Baumé hydrometer, preferably the former, as the scale graduations agree closely with percentages of total solids. A table for comparison of degrees Brix (density scale indicating directly percentage by weight of pure sucrose in pure solns), degrees Baumé (modulus 145), sp. gr. at 20/4°, and sp. gr. at 20/20° is given under 44.3.

Use spindle graduated in tenths, and as limited as possible in range of degrees recorded, and cylinder of sufficient diameter to permit spindle to come to rest without touching sides. Allow soln to come as nearly as practicable to same temp. as air at time of reading, and if this varies more than 1° from temp. at which spindle was graduated, 20°, apply correction according to 44.4. Before taking density of a juice, allow it to stand in cylinder until all air bubbles have escaped and all fatty or waxy matters have come to top and been skimmed off. (Air bubbles may be conveniently removed, especially in case of solns of high density, by applying vacuum to cylinder by means of tube passing thru stopper inserted in top of cylinder.) If sample is too dense to determine density directly, dilute weighed portion with weighed quantity of H<sub>2</sub>O, or dissolve weighed portion and dilute to known volume with H<sub>2</sub>O. In first instance, percentage of total solids is calculated by following formula:

Percentage of solids in undiluted material =  $WS/w$ , in which  $S$  = percentage of solids in diluted material;  $W$  = weight of diluted material; and  $w$  = weight of sample taken for dilution.

When dilution is made to definite volume, use following formula:

Percentage of solids in undiluted material =  $VDS/w$ , in which  $V$  = volume of diluted soln at given temp.;  $D$  = sp. gr. of diluted soln at same temp.;  $S$  = percentage of solids in diluted soln at same temp.; and  $w$  = weight of sample taken for dilution.

*Double dilution.*—The calculation is simplified by mixing equal weights of sugar product and  $H_2O$ , and multiplying Brix of soln by 2.

## 34.7

*By Means of Pycnometer (3)—Official*

(a) *Specific gravity (in vacuo or in air).*—Determine sp. gr. of soln at  $20/4^\circ$ ,  $20/20^\circ$  in vacuo, or  $20/20^\circ$  in air as directed under 16.4, and ascertain corresponding percentage by weight of sugar solids from appropriate table, 44.3 or 44.5. When density of substance is too high for direct determination, dilute and then calculate sucrose content of original material as directed under 34.6.

(b) *Specific gravity of molasses.*—Use special 100 ml volumetric flask with neck ca 8 mm inside diam. Weigh flask empty and then fill with molasses, using long-stemmed funnel reaching below graduation mark, until level of molasses is up to lower end of neck of flask. (Flow of molasses may be stopped by inserting glass rod of suitable size into funnel so as to close stem opening.) Remove funnel carefully to prevent molasses coming in contact with neck, and weigh flask and molasses. Add  $H_2O$  almost to graduation mark, running down side of neck to prevent mixing with the molasses. Allow to stand several hours or overnight to permit escape of bubbles. Place flask in constant temp. water bath, preferably at  $20^\circ$ , and leave until it reaches temp. of bath; then make to volume at that temp. with  $H_2O$ . Weigh. Reduce weight of molasses to vacuo and calculate density. Ascertain corresponding Brix or Baumé reading from 44.3.

*Example:*

	<i>grams</i>
A, wt $H_2O$ content flask $20^\circ$ in vacuo	= 99.823
B, wt molasses $20^\circ$ in vacuo	= 132.834
C, wt molasses and $H_2O$ $20^\circ$ in vacuo	= 137.968
$A - (C - B)$ = wt $H_2O$ occupying space of molasses in vacuo	= 94.689

$$\frac{132.834}{94.689} = 1.403 \text{ sp. gr. } \left( \frac{20^\circ}{20^\circ} \right) \text{ molasses.}$$

## 34.8

*By Means of Refractometer (4)—Official*

(Applicable only to liquid samples containing no undissolved solids)

Determine refractometer reading of soln at  $20^\circ$  and obtain corresponding percentage of dry substance from either the direct reading, if sugar refractometer is used, or from 44.7, if instrument gives readings in terms of refractive index. Circulate  $H_2O$  at constant temp., preferably  $20^\circ$ , thru jackets of refractometer or thru trough of immersion instrument, long enough to allow temp. of prisms and of sample to reach equilibrium, continuing circulation during observations and taking care that constant temp. is maintained. If determination is made at a temp. other than  $20^\circ$ , or if humidity causes condensation of moisture on exposed faces of prisms, make measurements at room temp. and correct readings to standard temp. of  $20^\circ$  according to 44.8. If soln is too dark to be read in the instrument, dilute with a concentrated

sugar soln; never use  $H_2O$  for this purpose. Mix weighed quantities of soln under examination and soln of pure sugar of about same strength, and obtain quantity of dry substance in former by following formula:

$$x = \frac{(A+B)C - BD}{A}, \text{ in which } x = \text{percentage of dry substance to be found;}$$

$A$  = weight (g) of sample mixed with  $B$ ;  $B$  = weight (g) of pure sugar soln used in dilution;  $C$  = percentage of dry substance in mixture  $A + B$  obtained from refractive index; and  $D$  = percentage of dry substance in the pure sugar soln obtained from its refractive index.

#### ASH—OFFICIAL

##### 34.9

##### *Method I*

Heat sample of appropriate weight for product being examined (usually 5–10 g) in 50–100 ml Pt dish at  $100^\circ$  until  $H_2O$  is expelled, add few drops of pure olive oil, and heat slowly over flame until swelling ceases. Place dish in muffle at ca  $525^\circ$  and leave until white ash is obtained. Moisten ash with  $H_2O$ , dry on steam bath and then on hot plate, and re-ash in muffle at  $525^\circ$  to constant weight.

##### 34.10

##### *Method II*

Carbonize sample of appropriate weight for product being examined (usually 5–10 g) in 50–100 ml Pt dish at ca  $525^\circ$  and treat charred mass with hot  $H_2O$  to dissolve soluble salts. (In case of low-purity products, addition of a few drops of pure olive oil, as in 34.9, may be desirable.) Filter thru ashless filter, ignite filter and residue to white ash, add filtrate of soluble salts, evaporate to dryness, and ignite at ca  $525^\circ$  to constant weight.

##### 34.11

##### *Sulfated Ash*

Weigh 5 g of sample into 50–100 ml Pt dish, add 5 ml of 10%  $H_2SO_4$ , ignite until sample is well carbonized, and then burn in muffle at ca  $550^\circ$ . Cool, add 2–3 ml of 10%  $H_2SO_4$ , evaporate on steam bath, dry on hot plate, and again ignite at  $550^\circ$  to constant weight. Express result as percentage of sulfated ash.

##### 34.12

##### MINERAL CONSTITUENTS—OFFICIAL.—See Chap. 12

##### 34.13

##### SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Ash the material as directed, 34.9 or 34.10. Add  $H_2O$  to ash in the Pt dish, heat nearly to boiling, filter thru ashless filter, and wash with hot  $H_2O$  until combined filtrate and washings measure ca 60 ml. Return filter and contents to Pt dish, ignite carefully, cool, and weigh. Calculate percentages of water-soluble and water-insoluble ash.

##### 34.14

##### ALKALINITY OF SOLUBLE ASH—OFFICIAL

Cool filtrate from 34.13 and titrate with 0.1  $N$   $HCl$ , 43.7 and 43.8, using methyl orange indicator, 6.3(f). Express alkalinity in terms of number of ml of normal acid/100 g of sample.

##### 34.15

##### ALKALINITY OF INSOLUBLE ASH—OFFICIAL

Add excess of 0.1  $N$   $HCl$  (usually 10–15 ml) to ignited insoluble ash in Pt dish, 34.13, heat to incipient boiling on asbestos plate, cool, and titrate excess of  $HCl$  with

0.1 *N* NaOH, using methyl orange indicator. Express alkalinity in terms of number of ml of normal acid/100 g of sample.

#### 34.16 MINERAL ADULTERANTS IN ASH (5)—TENTATIVE

In large porcelain evaporating dish, mix 100 g of sample with ca 35 g of  $\text{H}_2\text{SO}_4$  and evaporate to sirupy consistency. Pass electric current thru it while stirring by placing one Pt electrode in bottom of dish near one side and attaching other to lower end of glass rod with which contents are stirred. Begin with current of ca 1 ampere and gradually increase to 4. In 10–15 min., mass is reduced to fine dry char, which may be readily burned to white ash in original dish over free flame or in muffle.

This method is preferred to ordinary method of heating with  $\text{H}_2\text{SO}_4$ , especially in case of molasses, because, if properly manipulated, material comes quickly into form of very finely divided char or powder that is especially adapted for subsequent quick ignition.

If electric current is not available, treat in large porcelain dish 100 g of the saccharine soln, evaporate to sirupy consistency with sufficient  $\text{H}_2\text{SO}_4$  to carbonize mass thoroly, and ignite in usual manner.

Following adulterants may be present: salts of Sn, used in molasses to bleach; mineral pigments, such as  $\text{PbCrO}_4$  in yellow confectionery; oxide of Fe, sometimes used to simulate color of chocolate; and Cu. These elements may be detected by usual qualitative tests.

#### 34.17 NITROGEN—OFFICIAL

Determine N in 5 g of material as directed under 2.24, 2.25, or 2.26, using larger quantity of the  $\text{H}_2\text{SO}_4$  if necessary for complete digestion.

### SUCROSE—POLARIMETRIC METHODS

#### GENERAL PROCEDURE

#### 34.18 (a) *Directions for Raw Sugars—Official*

(Rules of International Commission for Uniform Methods of Sugar Analysis (6))

"In general all polarizations are to be made at 20°.

"Verification of saccharimeter must also be made at 20°. For instruments using Ventzke scale, dissolve 26 g of pure dry sucrose, weighed in air with brass weights, in  $\text{H}_2\text{O}$  so that 100 ml of soln is obtained at 20°. This soln, contained in 200 mm polariscope tube and polarized in room or cabinet, temp. of which is also 20°, must give saccharimeter reading of exactly 100.00. Temp. of the sugar soln during polarization must be kept constant at 20°."

According to determination of Bates and Jackson (7) at National Bureau of Standards, the Ventzke scale saccharimeter reading for 26 g of pure dry sucrose under above conditions is 99.895. The Ventzke scale reading has been redetermined by Balch and Hill (8) and by Zerban, Gamble, and Hardin (8), who found values of 99.907° and 99.912°, respectively. Average value of these three independent observers is 99.90. The value 99.90 was adopted by the International Commission in 1933. Most modern instruments are calibrated to read 100° and are designated "International Sugar Scale."

"For countries where mean temp. is higher than 20°, saccharimeters may be adjusted at 30° or any other suitable temp., under conditions specified above, provided sugar soln be diluted to final volume and polarized at this same temp.

"In determining polarization of substances containing sugar employ only half-shade instruments."

Saccharimeter used may have either single or double wedge and should be either 200 mm or 400 mm instrument.

"During observation keep apparatus in fixed position and so far removed from source of light that polarizing Nicol is not warmed.

"As sources of light, employ lamps which give strong illumination, such as triple gas burner with metallic cylinder, lens, and reflector; gas lamps with Auer (Welsbach) burner; electric lamp; petroleum duplex lamp; or Na light."

Whenever there is any irregularity in sources of light such as that due to convolutions of the filament in case of electric light or to meshes of gauze in the case of Welsbach light, place thin ground-glass plate between source of light and polariscope so as to render illumination uniform.

"Before and after each set of observations, chemist must satisfy himself of correct adjustment of his saccharimeter by means of standardized quartz plates. He must also previously satisfy himself of accuracy of his weights, polarization flasks, observation tubes, and cover-glasses. (Scratched cover-glasses must not be used.) Make several readings and take mean thereof, but no one reading may be rejected."

The quartz plates are standardized to read to second decimal place, and by their use a quick and at same time accurate test can be made. In using such plates for testing saccharimeters, it is necessary that the instrument, as well as plate, be at 20° before reading is made. Different points of the scale, preferably 20°, 50°, 80°, and 100° (sugar scale), should be tested against the plates. Scale may also be standardized by means of carefully calibrated telescopic control tube (9). A new type of telescopic control tube of high accuracy has recently been described by Zerban, Gamble, and Hardin (8).

"In determining the polarization use the whole normal weight for 100 ml or a multiple thereof for any corresponding volume.

"As clarifying and decolorizing agents use basic acetate of lead . . . , alumina cream, or concentrated soln of alum. Boneblack and decolorizing powders are to be excluded."

Whenever reducing sugars are determined in the soln for polarizing, use only neutral Pb acetate for clarification, as basic Pb acetate causes precipitation of some of the reducing sugars. In addition to clarifying agents mentioned, basic Pb nitrate has been made official by the Association.

"After bringing soln exactly to mark at proper temp., and after wiping out neck of flask with filter paper, pour all the well-shaken clarified sugar soln on rapidly acting, dry filter. Reject first portion of filtrate and use remainder, which must be perfectly clear, for polarization."

Cover funnel at the start of filtration. It is advisable to reject first 25 ml that runs thru, and use remainder for polarization. In no case should whole soln or any part be returned to filter. If cloudy after the 25 ml has been rejected, begin new determination.

"Whenever white light is used in polarimetric determinations, it must be filtered thru a soln of  $K_2Cr_2O_7$  of such concentration that percentage content of soln multiplied by length of column of soln in centimeters is equal to nine."

This concentration must be doubled in polarizing carbohydrate materials of high rotation dispersion, such as commercial glucose, etc.

#### (b) Conversion Factors of Different Saccharimeter Scales

1° Ventzke Scale	= 0.34657° Angular Rotation D
1° Angular Rotation D*	= 2.88542° Ventzke Scale
Normal weight Ventzke Scale	= 26.026 grams

\* Designation D refers to sodium light of 5893 Å Angström.

1° Bureau Standards Scale	=0.34620° Angular Rotation D
1° Angular Rotation D	=2.88850° Bureau Standards Scale
Normal weight Bur. Stand. Scale =26.000 grams	
1° Bidecimal Scale	=0.26622° Angular Rotation D
1° Angular Rotation D	=3.75629° Bidecimal Scale
Normal weight Bidecimal Scale =20.000 grams	
1° French Scale	=0.21667° Angular Rotation D
1° Angular Rotation D	=4.61538° French Scale
Normal weight French Scale =16.269 grams	

### 34.19 *Preparation and Use of Clarifying Reagents (10)—Official*

(a) *Basic lead acetate soln.*—Activate litharge by heating to 650–670° for 2.5–3 hours in muffle (cooled product should be lemon color). Boil 430 g of neutral Pb acetate, 130 g of freshly activated litharge, and 1 liter of H<sub>2</sub>O for 30 min. Allow mixture to cool and settle and then dilute supernatant liquid to sp. gr. of 1.25 with recently boiled H<sub>2</sub>O. Solid basic Pb acetate may be substituted for the normal salt and litharge in preparation of soln. (This reagent is used primarily for clarifying dark colored cane, sorghum, and beet products when sucrose is determined by polariscopic methods.)

(b) *Alumina cream.*—Prepare cold saturated soln of alum in H<sub>2</sub>O. Add NH<sub>4</sub>OH with constant stirring until soln is alkaline to litmus, allow precipitate to settle, and wash by decantation with H<sub>2</sub>O until wash H<sub>2</sub>O gives only slight test for sulfates with BaCl<sub>2</sub> soln. Pour off excess of H<sub>2</sub>O and store residual cream in stoppered bottle. (Alumina cream is suitable for clarifying light colored sugar products or as adjunct to other agents when sugars are determined by polariscopic or reducing sugar methods.)

(c) *Dry basic lead acetate.*—Obtained as dry powdered salt and should contain 72.8% of Pb, which corresponds to composition of 3Pb(C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>)<sub>2</sub>·2PbO. Of this salt, ca  $\frac{1}{3}$  g = 1 ml of basic Pb acetate soln described under (a). In making clarification, add small quantity of dry salt to sugar soln after completion to volume and shake; then add more salt and shake again, repeating addition until precipitation is complete, but avoiding any excess. When molasses or any other substance producing heavy precipitate is being clarified, add some dry, coarse sand to break up the pellets of basic Pb acetate and precipitate. (Dry basic Pb acetate can also be used in place of soln of this salt in clarifying cane, sorghum, and beet products.)

(d) *Neutral lead acetate.*—Prepare saturated soln of neutral Pb acetate and add to the sugar soln before completing to volume. (This reagent may be used for clarifying light-colored sugar products when sugars are determined by polariscopic methods, and its use is imperative when reducing sugars are determined in soln used for polarization.)

(e) *Basic lead nitrate.*—(1) Dissolve 250 g of Pb(NO<sub>3</sub>)<sub>2</sub> in H<sub>2</sub>O and make to 500 ml. (2) Dissolve 25 g of NaOH in H<sub>2</sub>O and make to 500 ml. In clarifying, add equal quantities of (1) and (2) to sugar soln, shake, and add more if complete precipitation has not occurred, but avoid excess. Complete the volume with H<sub>2</sub>O. (This reagent is used for same purposes as one described under (a).) When this soln is used for clarification, basic value of divisor in Clerget determination by acid becomes 143.5.

### 34.20 *Temperature Corrections for Polarization of Sugars (11)—Tentative*

(a) *Refined sugars.*—Polarizations of sugars testing 99 or above, when made at a

temp. other than 20°, may be calculated to polarizations at 20° by following formula:  
 $P_{20} = p'[1 + 0.0003(t - 20)]$ , in which  $p'$  = polarization at temp. read; and  $t$  = temp. at which polarization is read.

(b) *Raw cane sugars*.—Polarizations of raw cane sugars, when made at temps. other than 20°, may be calculated to polarizations at 20° by following formula:

$P_{20} = p' + 0.0015(p' - 80)(t - 20)$ , in which  $p'$  and  $t$  are same as in formula under (a).

When percentage of levulose in the sugar is known (which in case of honeys and sugar cane products is ca  $\frac{1}{2}$  the reducing sugars), following formula should be used:

$P_{20} = p' + 0.0003S(t - 20) - 0.00812L(t - 20)$ , in which  $p'$  and  $t$  are same as in formula under (a);  $S$  = percentage of sucrose; and  $L$  = percentage of levulose.

These formulas give results agreeing closely with polarizations obtained at 20° if the sugar is of average normal composition.

### 34.21

#### Mutarotation

Products, such as honey and commercial glucose, that contain dextrose or other reducing sugars in crystalline form or in soln at high density may show mutarotation under conditions prevailing during analysis. Only constant rotation should be used in polarimetric methods. To obtain this, allow soln prepared for polarization to stand overnight before making reading. If it is desired to make reading immediately, heat the neutral (pH ca 7.0) soln to boiling for a few moments or add a few drops of  $\text{NH}_4\text{OH}$  before completing to volume; or, if soln has been made to volume, add dry  $\text{Na}_2\text{CO}_3$  until just distinctly alkaline to litmus paper. (Do not allow the slightly alkaline solns to stand at such high temps., or for such lengths of time, as to cause destruction of levulose.) Determine completion of mutarotation by making readings at 15–30 min. intervals until these become constant.

### SUCROSE IN ABSENCE OF RAFFINOSE

#### *I. By Polarization Before and After Inversion with Invertase (12)—Official*

### 34.22

#### REAGENT

*Invertase soln*.—Commercial invertase preparations are available on the market. If it is desired to prepare the soln in the laboratory, procedure described under (1) may be used. In either case, preparation may be further purified and concentrated by ultrafiltration method described under (3). Commercial preparations may also be purified by dialysis and then reconcentrated by evaporating in vacuo at a temp. not exceeding 40°.

(1) *Crude invertase soln*.—Mix yeast with  $\text{H}_2\text{O}$  in proportion of 10 lbs. of compressed bakers' yeast to 5 liters of  $\text{H}_2\text{O}$ . Add 2 liters of toluene and stir thoroly at frequent intervals during first 24 hours. Allow to stand for 7 days with occasional stirring, and filter by gravity thru large fluted papers. Mix residue with 2 liters of  $\text{H}_2\text{O}$ , filter, and combine filtrates. Purify (13) by adding 15 g of neutral Pb acetate to each liter of extract and filtering on paper after all Pb acetate has been dissolved. Complete purification immediately by dialysis or by washing on ultrafilter as directed under (3).

(2) *Collodion ultrafilter (14)*.—Dissolve 6 g of soluble (in alcohol and ether mixture) pyroxylin or nitrocellulose such as Astoria's in mixture of 50 ml of absolute alcohol and 50 ml of absolute ether by first adding the alcohol to the cotton, allowing mixture to stand in stoppered flask for 10 min., adding the ether, and shaking. Allow soln to stand overnight, pour ca 100 ml into 2000 ml cylinder, and coat entire inside surface of cylinder with the collodion. Drain, and dry for 10 min. Fill



with  $\text{H}_2\text{O}$ , let stand 10–15 min., pour out the  $\text{H}_2\text{O}$ , and remove collodion sack. Test for leaks by filling with  $\text{H}_2\text{O}$ . Slit open longitudinally and cut out circular piece 7–8" in diam. Cut bottom from 2 liter bottle or Erlenmeyer flask and grind edge smooth. Place it upon the still moist collodion disk, fold edge of disk up around bottle, and cement it thereto with collodion that contains increased percentage of ether. Place 3 or 4 thicknesses of wet filter paper in 8" Büchner funnel. Place bottle with collodion membrane upon the filter paper. Pour melted vaseline, to depth of 1", between bottle and inside of funnel. Provide bottle with small mechanical stirring device.

(3) *Washing and concentration of invertase soln by ultrafiltration.*—Filter 4 liters of partially purified soln thru the ultrafilter, stirring continuously, until ca 1 liter remains. Wash with distilled  $\text{H}_2\text{O}$  introduced by means of constant level device until filtrate is colorless, 3 or 4 liters of wash  $\text{H}_2\text{O}$  being required. During entire process, invertase soln should be preserved with toluene.

(4) *Activity of invertase soln.*—Following test for activity of the invertase soln is usually adequate: Dilute 1 ml of the invertase preparation to 200 ml. Transfer 10 g of sucrose (granulated sugar) to sugar flask graduated at 100 ml and 110 ml, dissolve in ca 76 ml of  $\text{H}_2\text{O}$ , add 2 drops of acetic acid, and dilute to 100 ml mark. To the 100 ml of sugar soln add 10 ml of the dilute invertase soln and mix thoroly and rapidly, noting exact time at which solns are mixed. At termination of exactly 60 min. make portion of soln just distinctly alkaline to litmus paper with anhydrous  $\text{Na}_2\text{CO}_3$  and polarize in 200 mm tube at  $20^\circ$ . If invertase soln is sufficiently active, the alkaline soln will polarize ca  $31^\circ$  Ventzke without correcting for the dilution to 110 ml and the optical activity of the invertase soln.

If more exact information concerning the activity of the invertase preparation is desired, determine its velocity constant as follows: Dilute 1 ml of the invertase soln to 200 ml at  $20^\circ$ ; place in constant temp. bath at  $20^\circ$ ; and when soln has attained the latter temp., pipet 20 ml of it into flask containing 200 ml of a sucrose soln (10 g/100 ml concentration) that has been previously made distinctly acid to methyl red (corresponding to pH ca 4.6 (15)) by addition of acetic acid and also brought to temp. of  $20^\circ$  in same bath. Mix thoroly and promptly and note time at which invertase soln was added. Keep sucrose-invertase mixture in constant temp. bath; remove portions at end of 15, 30, and 45 min.; render each portion just distinctly alkaline to litmus paper with anhydrous  $\text{Na}_2\text{CO}_3$  immediately after removing; and polarize at  $20^\circ$ . Correct all polarizations for polarization of invertase soln. Calculate velocity constant,  $k$ , for each of polarizations (at the time  $t$ ) subsequent to initial polarization by following formula:

$$k = \frac{\log_{10} 1.32 R_0 - \log_{10} (R_t + 0.32 R_0)}{t}, \text{ in which } k = \text{unimolecular reaction velocity}$$

constant;  $t$  = number of minutes elapsing from time invertase and sucrose solns were mixed until inversion was stopped by addition of  $\text{Na}_2\text{CO}_3$ ;  $R_0$  = initial polarization (calculated by multiplying polarization of sucrose soln by 10/11 and correcting for polarization of invertase soln); and  $R_t$  = polarization at time  $t$ .

An invertase soln of sufficient activity (16) should yield average value for  $k$  (for various time periods) of at least 0.1 after multiplying the  $k$  value directly obtained by 200, in order to correct for initial dilution of invertase soln. Dilution of invertase soln above mentioned is made solely for purpose of determining its activity; original, undiluted invertase soln is used as inverting reagent in determination of sucrose, 34.23, unless activity of original invertase soln greatly exceeds  $k$  value of 0.1, and it is desirable to conserve the invertase. In this case, dilute to  $k$  value of 0.1, which

is done in same manner as diluting other solns to standard strength. Activity of invertase preparation required for rapid inversion, 34.23(c), is same as that needed for overnight inversion, 34.23(b), but proportion of invertase preparation used in former case is twice that used in latter instance.

## 34.23

## DETERMINATION

(a) *Direct reading*.—Dissolve double normal weight of the substance (52 g), or fraction thereof, in H<sub>2</sub>O in 200 ml volumetric flask; add necessary clarifying agent, 34.19(a), (b), (d), or (e), avoiding any excess; shake, dilute to mark with H<sub>2</sub>O, mix well, and filter, keeping funnel covered with watch-glass. Reject first 25 ml of filtrate. If a Pb clarifying agent was used, remove excess Pb from soln when sufficient filtrate has collected by adding anhydrous Na<sub>2</sub>CO<sub>3</sub>, a little at a time, avoiding any excess; mix well and filter again, rejecting first 25 ml of filtrate. (Instead of weighing 52 g into 200 ml flask, two 26 g portions may be diluted to 100 ml each, and treated exactly as described. Depending on color of product, multiples or fractions of normal weight may be used, and results reduced by calculation to basis of 26 g in 100 ml.) Pipet one 50 ml portion of Pb-free filtrate into 100 ml flask, dilute with H<sub>2</sub>O to mark, mix well, and polarize in 200 mm tube. Result, multiplied by 2, is direct reading (*P* of formula given below) or polarization before inversion. (If 400 mm tube is used, reading equals *P*.) If there is a possibility of mutarotation, proceed as directed under 34.21.

(b) *Invert reading*.—First determine quantity of acetic acid necessary to render 50 ml of the Pb-free filtrate distinctly acid to methyl red indicator; then to another 50 ml of the Pb-free soln in 100 ml volumetric flask, add requisite quantity of acid and 5 ml of the invertase preparation, fill flask with H<sub>2</sub>O nearly to 100 ml, and let stand overnight (preferably at not less than 20°). Cool, and dilute to 100 ml at 20°. Mix well and polarize at 20° in 200 mm tube. If in doubt as to completion of hydrolysis, allow a portion of soln to remain for several hours and again polarize. If there is no change from previous reading, inversion is complete. Carefully note reading and temp. of soln. If it is necessary to work at temp. other than 20°, which is permissible within narrow limits, complete volumes and make both direct and invert readings at same temp. Correct polarization for optical activity of invertase soln and multiply by 2. Calculate percentage of sucrose by following formula:

$$S = \frac{100 (P - I)}{142.1 + 0.073 (m - 13) - t/2}, \text{ in which } S = \text{percentage of sucrose; } P = \text{direct reading, normal soln; } I = \text{invert reading, normal soln; } t = \text{temp. at which readings are made; and } m = \text{g of total solids from original sample in 100 ml of the invert soln. Determine total solids as percentage by weight, as directed under 34.8, and multiply this figure by density at 20°, 44.3.}$$

ing, normal soln; *I* = invert reading, normal soln; *t* = temp. at which readings are made; and *m* = g of total solids from original sample in 100 ml of the invert soln. Determine total solids as percentage by weight, as directed under 34.8, and multiply this figure by density at 20°, 44.3.

(c) *Rapid inversion at 55–60° (17)*.—If more rapid inversion is desired, proceed as follows: Prepare sample as directed under (a) and to 50 ml of Pb-free filtrate in 100 ml volumetric flask add acetic acid in sufficient quantity to render soln distinctly acid to methyl red, 34.22(4). Quantity of acetic acid required should be determined before pipetting the 50 ml portion as directed under (b). Add 10 ml of invertase soln, mix thoroly, place flask in water bath at 55–60°, and allow to stand at that temp. for 15 min. with occasional shaking. Cool, add Na<sub>2</sub>CO<sub>3</sub> until distinctly alkaline to litmus paper, dilute to 100 ml at 20°, mix well, and determine polarization at 20° in 200 mm tube. Allow soln to remain in tube for 10 min. and again determine polarization. If there is no change from previous reading, mutarotation is

complete. Carefully note reading and temp. of soln. Correct polarization for optical activity of the invertase soln and multiply by 2. Calculate percentage of sucrose by formula given under (b).

If soln has been rendered so alkaline as to cause destruction of sugar, the polarization, if negative, will in general decrease, since decomposition of levulose ordinarily is more rapid than that of other sugars present. If soln has not been made sufficiently alkaline to complete mutarotation quickly, the polarization, if negative, will in general increase. As the analyst gains experience he may omit the polarization after 10 min. if he has satisfied himself that he is adding  $\text{Na}_2\text{CO}_3$  in sufficient amount to complete mutarotation at once without causing any destruction of sugar during period intervening before polarization.

### 34.24 II. By Polarization Before and After Inversion with Hydrochloric Acid (18)—Official

(In presence of much levulose, as in honeys, fruit products, sorghum sirup, cane sirup, and molasses, the optical method for sucrose, requiring hydrolysis by acid, gives erroneous results.)

(a) *Direct reading*.—Proceed as directed under 34.23(a).

(b) *Invert reading*.—Pipet 50 ml portion of Pb-free filtrate into 100 ml flask and add 25 ml of  $\text{H}_2\text{O}$ . Add, little by little, while rotating flask, 10 ml of  $\text{HCl}$  (sp. gr. 1.1029 at  $20/4^\circ$  or  $24.85^\circ$  Brix at  $20^\circ$ ). Heat water bath to  $70^\circ$  and regulate burner so that temp. of bath remains approximately at that point. Place flask in water bath, insert thermometer, and heat with constant agitation until thermometer in flask indicates  $67^\circ$ . (This preliminary heating period should require  $2\frac{1}{2}$ – $2\frac{3}{4}$  min.) From the moment thermometer in flask indicates  $67^\circ$ , leave flask in bath exactly 5 min. longer, during which time temp. should gradually rise to ca  $69.5^\circ$ . Plunge flask at once into  $\text{H}_2\text{O}$  at  $20^\circ$ . When contents have cooled to ca  $35^\circ$ , remove thermometer from flask, rinse it, and fill almost to mark. Leave flask in bath at  $20^\circ$  at least 30 min. longer and finally make up exactly to volume. Mix well and polarize soln in 200 mm tube provided with lateral branch and water jacket, maintaining temp. of  $20^\circ$ . This reading must also be multiplied by 2 to obtain the invert reading. If it is necessary to work at a temp. other than  $20^\circ$ , which is permissible within narrow limits, volumes must be completed and both direct and invert polarizations must be made at exactly same temp.

Calculate sucrose by following formula:

$$S = \frac{100(P - I)}{143 + 0.0676(m - 13) - t/2}, \text{ in which } S = \text{percentage of sucrose; } P = \text{direct reading, normal soln; } I = \text{invert reading, normal soln; } t = \text{temp. at which readings are made; and } m = \text{g of total solids from original sample in 100 ml of invert soln.}$$

Determine total solids as percentage by weight, as directed under 34.8, and multiply this figure by density at  $20^\circ$ , 44.3.

Determine total solids as percentage by weight, as directed under 34.8, and multiply this figure by density at  $20^\circ$ , 44.3.

(c) *Inversion at room temperature*.—Inversion may also be accomplished as follows: (1) To 50 ml of clarified soln, freed from Pb, add 10 ml of  $\text{HCl}$  (sp. gr. 1.1029 at  $20/4^\circ$  or  $24.85^\circ$  Brix at  $20^\circ$ ) and set aside for 24 hours at temp. not below  $20^\circ$ ; or, (2) if temp. is above  $25^\circ$ , set aside for 10 hours. Make up to 100 ml at  $20^\circ$  and polarize as directed under (b). Under these conditions formula must be changed to

$$\text{following: } S = \frac{100(P - I)}{143.2 + 0.0676(m - 13) - t/2}$$

## SUCROSE AND RAFFINOSE (13)

*I. By Polarization Before and After Treatment with Two Enzyme Preparations—Official*

## 34.25

## REAGENTS

(a) *Invertase soln (top yeast extract).*—Prepare as directed under 34.22. This soln should be free from the enzyme melibiase. Its invertase activity should be at least as great as that used for determination of sucrose in the absence of raffinose, 34.22 (4).

(b) *Invertase-melibiase soln (bottom yeast extract).*—Prepare as directed under 34.22, using bottom fermenting yeast (brewers' yeast) instead of bakers' yeast. Invertase activity should be at least as great as in (a).

Test melibiase activity of soln as follows: Add 2 ml of soln to be tested to 20 ml of weakly acid melibiose soln polarizing  $+20.0^{\circ}\text{V}$  and allow to stand 30 min. at ca  $20^{\circ}$ . Add sufficient  $\text{Na}_2\text{CO}_3$  to render soln slightly alkaline to litmus paper. Preparation suitable for overnight hydrolysis of solns containing not more than 0.2 g of raffinose in 100 ml should have hydrolyzed 35% of the melibiose present under conditions mentioned; preparation suitable for overnight hydrolysis of solns containing not more than 0.65 g of raffinose in 100 ml should have produced 50% hydrolysis of melibiose; and preparation suitable for overnight hydrolysis of solns containing 0.65–1.3 g of raffinose in 100 ml should have hydrolyzed at least 70% of melibiose present under above condition. Polarizations that correspond to 35, 50 and 70% hydrolysis of a melibiose soln polarizing, before hydrolysis,  $+20^{\circ}\text{V}$  are:  $+16.4^{\circ}$ ,  $+14.9^{\circ}$ , and  $+12.9^{\circ}\text{V}$ , respectively.

## 34.26

## DETERMINATION

In analyzing sugar beet products, weigh quantity of material specified in

34.27 *Quantities of sample and reagents required for clarification and deleading of beet sugar-house products*

MATERIAL	QUANTITY PER 100 ML	BASIC LEAD ACETATE (55° BRIX)	AMMONIUM DIHYDROGEN PHOSPHATE
	grams	ml	gram
Cossettes <sup>a</sup> . . . . .	13	3	0.2
Pulp . . . . .	100 ml <sup>b</sup>	2–4	0.2
Lime cake or sewer <sup>c</sup> . . . . .	26.5	1.5	. . . . <sup>d</sup>
Thin juice . . . . .	52	2	0.2–0.3
Thick juice . . . . .	26	4	0.3–0.4
White massecuite . . . . .	13 or 26	3 or 6	0.3–0.7
High wash sirup . . . . .	13 or 26	3 or 6	0.3–0.7
High green sirup . . . . .	13 or 26	5 or 10	0.3–0.7
Raw or remelt massecuite . . . . .	13	6	0.3–0.4
Raw or remelt sugar . . . . .	26	3–4	0.3–0.4
Sugar melter . . . . .	26	2–3	0.3–0.4
Low wash sirup . . . . .	13	8–10	0.4–0.5
Low green sirup or molasses . . . . .	13	10	0.4–0.5
Saccharate cakes and milk (carbonated) . . . . .	26	4–6	0.3–0.4
Steffen waste and wash waters <sup>e</sup> . . . . .	78 or 50 ml	2–3	0.2

<sup>a</sup> Usual method of extraction, 26 g in 201.2 ml.

<sup>b</sup> Dilute to 110 ml.

<sup>c</sup> Neutralize with acetic acid before adding basic Pb acetate.

<sup>d</sup> Lime in soln will be partly precipitated by the phosphate, and it is necessary to add sufficient phosphate to complete the precipitation of both Pb and Ca salts; hence no definite quantity can be specified.

table, 34.27, transfer to 300 ml volumetric flask, add quantity of basic Pb acetate soln indicated in table, and dilute to volume at 20°. Mix thoroly and filter thru fluted paper in closely covered funnel, rejecting first 25 ml of filtrate. When sufficient filtrate has collected, remove Pb from soln by adding  $\text{NH}_4\text{H}_2\text{PO}_4$  in as small excess as possible, 34.27. This condition is readily determined after a little practice by appearance of the  $\text{Pb}_3(\text{PO}_4)_2$  precipitate, which usually flocculates and settles rapidly in presence of slight excess of the salt. Mix well and filter, again rejecting at least the first 25 ml of filtrate. Make a direct polarization in 200 mm tube at 20° unless soln contains appreciable quantity of invert sugar, in which case pipet 50 ml portion of Pb-free filtrate into 100 ml flask, dilute with  $\text{H}_2\text{O}$  to mark, mix well, and polarize at 20°, preferably in 400 mm tube. This reading, calculated to normal weight of 26 g in 100 ml and 200 mm tube length, is the direct reading ( $P$ ) of formula given below for polarization before inversion.

Transfer two 50 ml portions of the Pb-free filtrate to 100 ml flasks. To one add 5 ml of invertase soln (top yeast extract) and to the other 5 ml of invertase-melibiose soln (bottom yeast extract), let stand overnight at room temp. (preferably not below 20°), dilute to volume, mix well, and polarize at 20°, preferably in 400 mm jacketed tube. If rapid hydrolysis is desired, add 10 ml of each of the enzyme solns to the 50 ml portions of delead filtrate in 100 ml flasks and place in water bath at 50–55° for 40 min. Then add  $\text{Na}_2\text{CO}_3$  until soln is slightly alkaline to litmus paper, dilute to volume at 20°, mix well, and polarize at 20°, preferably in 400 mm tube. Correct invert readings for optical activity of enzyme soln and calculate polarization to that of a normal weight soln of 26 g in 100 ml; also calculate reading to 200 mm tube length, if necessary.

Calculate percentages of anhydrous raffinose and sucrose from following formulas:

$$R = 1.354(A - B); S = \frac{(P - 2.202A + 1.202B)100}{132.12 - 0.00718[132.12 - (P - 2.202A + 1.202B)]}, \text{ in which}$$

$R$  = percentage of raffinose;  $S$  = percentage of sucrose;  $P$  = direct polarization, normal soln;  $A$  = corrected polarization after top yeast hydrolysis, normal soln; and  $B$  = corrected polarization after bottom yeast hydrolysis, normal soln.

The quantities  $A$  and  $B$  are treated algebraically.

### 34.28 II. By Polarization Before and After Inversion with Hydrochloric Acid—Official

(Of value chiefly in analysis of beet products)

If direct reading is more than 1° higher than percentage of sucrose as calculated by formula given under 34.24(b), raffinose is probably present. Calculate sucrose and raffinose by following formulas (20):

When polarizations are made at 20°:

$$S = \frac{0.514P - I}{0.844} \text{ and } R = \frac{0.33P + I}{1.563}, \text{ in which}$$

$P$  = direct reading, normal soln;  $I$  = invert reading, normal soln;  $S$  = percentage of sucrose; and  $R$  = percentage of anhydrous raffinose.

Following formulas (20) are applicable at all temps. other than 20°:

$$S = \frac{P(0.478 + 0.0018t_1) - I(1.006 - 0.0003t_1)}{(0.908 - 0.0032t_2)(1.006 - 0.0003t_1)}, \text{ and}$$

$$R = \frac{P(0.43 - 0.005t_2) + I(1.006 - 0.0003t_1)}{(1.681 - 0.0059t_2)(1.006 - 0.0003t_1)}, \text{ in which}$$

$P$  = direct reading, normal soln;  $I$  = invert reading, normal soln;  $S$  = percentage of sucrose;  $R$  = percentage of anhydrous raffinose;  $t_1$  = temp. of direct polarization; and  $t_2$  = temp. of invert polarization.

### 34.29 SUCROSE BY DOUBLE DILUTION METHOD (#1)—OFFICIAL

(Substances in which volume of combined insoluble matter and precipitate from clarifying agents is more than 1 ml from 26 g)

Weigh half-normal weight of sample and dilute soln to 100 ml, using appropriate clarifier (basic Pb acetate for dark colored confectionery or molasses and alumina cream for light colored confectionery). Also weigh a normal weight of sample and dilute second soln with the clarifier to 100 ml. Filter, and obtain direct polariscopic readings of both solns. Invert each soln as directed under 34.23(b) or (c) or 34.24(b) or (c) and obtain respective invert readings.

True direct polarization of sample = 4 times direct polarization of diluted soln less direct polarization of undiluted soln. True invert polarization = 4 times invert polarization of diluted soln less invert polarization of undiluted soln. Calculate sucrose from true polarizations thus obtained, using formula under 34.23 or 34.24 corresponding to method of inversion used.

### SUCROSE—CHEMICAL METHODS

#### 34.30 *From Reducing Sugars Before and After Inversion—Official*

Determine reducing sugars (clarification having been effected with neutral Pb acetate, never with basic Pb acetate) as directed under 34.40 and calculate to invert sugar from 44.11 or 44.12. Invert soln as directed under 34.23(b) or (c), or 34.24(b) or (c); exactly neutralize the acid; and again determine reducing sugars, but calculate them to invert sugar from tables referred to above, using invert sugar column alone. Deduct percentage of invert sugar obtained before inversion from that obtained after inversion and multiply difference by 0.95 to obtain percentage of sucrose. Dilute the solns in both determinations so that not more than 230 mg of invert sugar is present in quantity taken for reduction. It is important that all Pb be removed from soln with anhydrous powdered K oxalate before reduction.

### COMMERCIAL GLUCOSE (#2) (APPROXIMATE)—POLARIMETRIC METHODS—TENTATIVE

#### 34.31 *Method I. (Substances containing little or no invert sugar)*

Commercial glucose cannot be determined accurately owing to varying quantities of dextrin, maltose, and dextrose present in the product. However, in sirups in which quantity of invert sugar is so small as not to affect appreciably the result, commercial glucose may be estimated approximately by following formula:

$$G = \frac{(a - S)100}{211}, \text{ in which } G = \text{percentage of commercial glucose solids; } a = \text{direct}$$

polarization, normal soln; and  $S$  = percentage of cane sugar.

Express results in terms of commercial glucose solids polarizing +211°V. (This result may be recalculated in terms of commercial glucose of any Baumé reading desired.)

**34.32**                      *Method II. (Substances containing invert sugar)*

Prepare inverted half-normal soln of substance as directed under 34.24(b), except to cool soln after inversion, make neutral to phenolphthalein with NaOH soln, slightly acidify with HCl (1+5), and treat with 5–10 ml of alumina cream before making up to mark. Filter, and polarize at 87° in 200 mm jacketed metal tube. Multiply reading by 200 and divide by factor 196 to obtain quantity of commercial glucose solids polarizing +211°V. (This result may be recalculated in terms of commercial glucose of any Baumé reading desired.)

**INVERT SUGAR—CHEMICAL METHODS***I. Lane-Eynon General Volumetric Method (23)—Official***34.33****REAGENTS**

*Soxhlet modification of Fehling soln.*—Prepare by mixing immediately before use equal volumes of (a) and (b).

(a) *Copper sulfate soln.*—Dissolve 34.639 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ , dilute to 500 ml, and filter thru prepared asbestos. Determine Cu content of soln (preferably by electrolysis, 34.45) and so adjust that it will contain 440.9 mg of Cu/25 ml.

(b) *Alkaline tartrate soln.*—Dissolve 173 g of Rochelle salt and 50 g of NaOH in  $\text{H}_2\text{O}$ , dilute to 500 ml, allow to stand 2 days, and filter thru prepared asbestos.

(c) *Standard invert sugar soln.*—To soln of 9.5 g of pure sucrose add 5 ml of HCl and dilute with  $\text{H}_2\text{O}$  to ca 100 ml. Let stand several days at room temp. (ca 7 days at 12–15° or 3 days at 20–25°), then dilute to 1 liter. (Acidified 1% invert sugar soln is very stable over period of several months.) Neutralize with NaOH and dilute to desired concentration immediately prior to use.

**34.34****STANDARDIZATION AND METHOD OF TITRATION**

Pipet accurately 10 or 25 ml of mixed Soxhlet reagent or pipet 5 or 12.5 ml of each of Soxhlet solns (a) and (b) into flask of 300–400 ml capacity. Quantity of Cu taken will differ slightly between the two methods of pipetting, and method used must be carried out consistently during standardization and determination. Prepare standard soln of the pure sugar of such concentration that more than 15 ml and less than 50 ml will be required to reduce all the Cu. Titer may be calculated as follows: factor/mg sugar in 1 ml. Add almost the whole of the sugar soln required to effect reduction of all the Cu, so that not more than 0.5–1 ml is required later to complete titration. Heat the cold mixture to boiling on wire gauze and maintain in moderate ebullition for 2 min., lowering flame sufficiently to avoid bumping. Without removing flame add 2–5 drops of 1% aqueous methylene blue soln and complete titration within total boiling time of ca 3 min. by small additions of sugar soln to decolorization of indicator.

Multiply titer by number of mg in 1 ml of the standard soln to obtain the factor. Compare with tabulated factor to determine correction, if any, to be applied to table. Small deviations from tabulated factors may arise from variations in individual procedure or composition of reagents. If only approximate results (within 1%) are required, the standardization may be omitted, provided specifications of the analysis are rigidly observed.

**34.35****DETERMINATION**

If approximate concentration of sugar in sample is unknown, proceed by incremental method of titration. Add to 10 or 25 ml of Soxhlet soln 15 ml of the sugar

soln and heat to boiling over wire gauze. Boil ca 15 seconds and add rapidly further quantities of the sugar soln until only faintest perceptible blue color remains. Then add 2-5 drops of methylene blue and complete titration by adding the sugar soln dropwise. (Error resulting from this titration will not generally exceed 1%.)

For higher precision repeat titration, adding almost the whole of the sugar soln required to reduce all the Cu and proceed as directed above. In 44.19 or 44.20 find factor corresponding to titer and apply correction previously determined. Estimate as follows:  $\text{factor} \times 100 / \text{titer} = \text{mg of sugar in 100 ml.}$

### II. Scales Method (24)—Tentative

(Suitable when very small quantities of sugar are present)

#### 34.36

##### REAGENT

*Benedict soln.*—Dissolve 16 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 125-150 ml of  $\text{H}_2\text{O}$ . Dissolve 150 g of Na citrate, 130 g of  $\text{Na}_2\text{CO}_3$  (anhydrous), and 10 g of  $\text{NaHCO}_3$  in ca 650 ml of  $\text{H}_2\text{O}$ , heating to accelerate soln. Cool and combine the two solns with stirring, make to 1 liter, and filter.

#### 34.37

##### DETERMINATION

Transfer 20 ml of the Cu reagent to 300 ml Erlenmeyer flask fitted with 2-holed rubber stopper. Add 10 ml of sugar soln containing less than 20 mg of reducing sugar. Place over flame, bring to boiling in 4 min., and continue boiling exactly 3 min. (Approximate conditions, flame 50 mm, cone 20 mm, asbestos gauze 30 mm above burner. If preferred, electric heater may be used.) At expiration of 3 min. from beginning of boiling, cool rapidly by holding under cold water faucet, and add 100 ml of acetic acid soln (24 ml of acetic acid/liter) from graduate, and an exactly measured amount of 0.04 N I. Add 25 ml of HCl (60 ml/liter) from pipet held against side of flask, and agitate to distribute the acid rapidly. Rotate flask 1 min., or until all precipitate is dissolved. Titrate excess I with 0.04 N  $\text{Na}_2\text{S}_2\text{O}_3$ , using starch soln as indicator.

For amounts less than 20 mg of sugar each ml of I soln consumed will represent a constant quantity of sugar; for dextrose, ca 1.12 mg/ml. (For accurate work, analyst should determine conversion factor for particular conditions under which he is working by using control solns of the pure sugars under examination.)

### III. Munson-Walker General Method (25)

#### 34.38

##### REAGENTS

*Asbestos.*—Digest asbestos, which should be amphibole variety, with HCl (1+3) for 2-3 days. Wash free from acid, digest for similar period with 10% NaOH soln, and then treat for a few hours with hot alkaline tartrate soln (old alkaline tartrate solns that have stood for some time may be used for this purpose) of strength used in sugar determinations. Wash asbestos free from alkali; digest for several hours with  $\text{HNO}_3$  (1+3); and, after washing free from acid, shake with  $\text{H}_2\text{O}$  into fine pulp. In preparing a Gooch crucible, make a film of asbestos  $\frac{1}{8}$ " thick and wash thoroly with  $\text{H}_2\text{O}$  to remove fine particles of asbestos. If the precipitated  $\text{Cu}_2\text{O}$  is to be weighed as such, wash crucible with 10 ml of alcohol, then with 10 ml of ether; dry for 30 min. at  $100^\circ$ , cool in desiccator, and weigh.

The other reagents and solns used are described under 34.33. Clarification may be effected by neutral Pb acetate soln, 34.19(d) (never basic Pb acetate). Remove excess Pb with dry  $\text{Na}_2\text{C}_2\text{O}_4$ .



## 34.39

## PRECIPITATION OF CUPROUS OXIDE

Transfer 25 ml of each of the  $\text{CuSO}_4$  and alkaline tartrate solns to 400 ml beaker of alkali-resistant glass and add 50 ml of the reducing sugar soln, or if smaller volume of sugar soln is used, add  $\text{H}_2\text{O}$  to make final volume 100 ml. Heat beaker on asbestos gauze over Bunsen burner, regulate flame so that boiling begins in 4 min., and continue boiling for exactly 2 min. (It is important that these directions be strictly observed. To regulate burner for this purpose it is advisable to make preliminary tests, using 50 ml of reagent and 50 ml of  $\text{H}_2\text{O}$  before proceeding with actual determination. Electric heater may be used instead of burner.) Keep beaker covered with watch-glass during heating. Filter hot soln at once thru asbestos mat in porcelain Gooch crucible, using suction. Wash precipitate of  $\text{Cu}_2\text{O}$  thoroly with  $\text{H}_2\text{O}$  at ca  $60^\circ$  and either weigh directly as  $\text{Cu}_2\text{O}$ , 34.40, or determine quantity of reduced Cu by one of methods described under 34.41–34.45, respectively. Conduct blank determination, using 50 ml of the reagent and 50 ml of  $\text{H}_2\text{O}$ , and if weight of  $\text{Cu}_2\text{O}$  obtained exceeds 0.5 mg, correct result of reducing sugar determination accordingly. The alkaline tartrate soln deteriorates on standing, and the quantity of  $\text{Cu}_2\text{O}$  obtained in the blank increases.

## DETERMINATION OF REDUCED COPPER

## 34.40

*By Direct Weighing of Cuprous Oxide—Official*

(Use only for determinations in solns of reducing sugars of comparatively high purity. In products containing large quantities of mineral or organic impurities, including sucrose, determine the Cu of the  $\text{Cu}_2\text{O}$  by one of methods described under 34.41–34.45, since the  $\text{Cu}_2\text{O}$  is likely to be contaminated with foreign matter.)

Prepare a Gooch crucible as directed under 34.38. Collect precipitated  $\text{Cu}_2\text{O}$  on mat as directed under 34.39, and wash thoroly with hot  $\text{H}_2\text{O}$ , then with 10 ml of alcohol, and finally with 10 ml of ether. Dry precipitate for 30 min. in oven at  $100^\circ$ , cool, and weigh. Obtain from 44.11, weight of invert sugar equivalent to weight of  $\text{Cu}_2\text{O}$ .

Number of mg of  $\text{Cu}_2\text{O}$  reduced by given quantity of reducing sugar varies, depending upon whether or not sucrose is present. In the table absence of sucrose is assumed except in the entries under invert sugar, where, in addition to column for invert sugar alone, one column is given for mixtures of invert sugar and sucrose containing 0.4 g of total sugar in 50 ml of soln and one column for invert sugar and sucrose when the 50 ml of soln contains 2 g of total sugar. Two entries are also given under lactose and sucrose mixtures, showing proportions of 1 part lactose to 4 and 12 parts of sucrose, respectively.

*By Titration with Sodium Thiosulfate (28)—Official*

## 34.41

## . REAGENT

*Standard thiosulfate soln.*—Prepare soln containing 39 g of pure  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in 1 liter. Weigh accurately 0.2–0.4 g of pure electrolytic Cu and transfer to 250 ml Erlenmeyer flask roughly graduated by marks at 20 ml intervals. Dissolve the Cu in 5 ml of mixture of equal volumes of  $\text{HNO}_3$  and  $\text{H}_2\text{O}$ , dilute to 20 or 30 ml, boil to expel red fumes, add slight excess of strong Br water, and boil until Br is completely driven off. Cool, and add 10 ml of Na acetate soln (574 g of trihydrate/liter). Prepare soln of 42 g of KI in 100 ml of soln made very slightly alkaline to avoid formation and oxidation of HI. Add 10 ml of the KI soln and titrate the  $\text{Na}_2\text{S}_2\text{O}_3$  to

end point. Add 2 g of  $\text{NH}_4\text{SCN}$  (27) and stir until completely dissolved. Continue titration until precipitate is perfectly white.

It is essential for the  $\text{Na}_2\text{S}_2\text{O}_3$  titration that concentration of KI in soln be carefully regulated. If soln contains less than 320 mg of Cu, at completion of titration 4.2–5 g of KI should have been added for each 100 ml of total soln. If greater quantities of Cu are present, add the KI soln slowly from buret with constant agitation in amounts proportionately greater.

Observe volume of the Cu soln and add 1 ml of KI soln for each 10 ml of the soln undergoing titration. Titrate at once with the  $\text{Na}_2\text{S}_2\text{O}_3$  soln until the brown color becomes faint. Again observe volume and add an additional volume of KI to make required concentration, noting from volume of  $\text{Na}_2\text{S}_2\text{O}_3$  soln the approximate Cu content of soln. Add sufficient starch indicator, 6.3(c), to produce a marked blue coloration. Continue titration cautiously until color changes toward end to faint lilac. As end point is approached, add the  $\text{Na}_2\text{S}_2\text{O}_3$  in fractions of drops, allowing precipitate to settle slightly after each addition. 1 ml of the  $\text{Na}_2\text{S}_2\text{O}_3$  soln = ca 10 mg of Cu.

#### 34.42

#### DETERMINATION

Wash the precipitated  $\text{Cu}_2\text{O}$ , cover the Gooch crucible with watch-glass, and dissolve the oxide by means of 5 ml of  $\text{HNO}_3$  (1+1) directed under watch-glass with pipet. Collect filtrate in 250 ml Erlenmeyer flask, which is roughly graduated by marks at 20 ml intervals, and wash watch-glass and Gooch crucible free from Cu. Proceed as directed under 34.41, beginning, "boil to expel red fumes . . ." Obtain weight of reducing sugar equivalent to weight of Cu from 44.12.

#### *By Titration with Potassium Permanganate (28)—Tentative*

#### 34.43

#### REAGENTS

(a) *Potassium permanganate soln.*—Approximately 0.1573 *N*, and containing 4.98 g/liter. After several days' aging, filter thru asbestos or sintered glass. Standardize by one of following methods:

(1) Transfer 0.35 g of  $\text{Na}_2\text{C}_2\text{O}_4$  (dried at  $103^\circ$ ) to 600 ml beaker. Add 250 ml of  $\text{H}_2\text{SO}_4$  (5+95) previously boiled 10 min. and cooled to  $27^\circ \pm 3^\circ$ . Stir until oxalate is dissolved. Add 29–30 ml of the  $\text{KMnO}_4$  soln at rate of 25–35 ml/min. while stirring slowly. Allow mixture to stand until pink color disappears (ca 45 seconds). Heat to  $55\text{--}60^\circ$ , and complete titration by adding  $\text{KMnO}_4$  until faint pink color persists 30 seconds. Add last 0.5–1 ml dropwise, allowing each drop to become decolorized before adding the next.

Determine excess of soln (usually 0.03–0.05 ml) required to impart pink color to same volume of acid boiled and cooled to  $55\text{--}60^\circ$ . (In potentiometric titrations correction is negligible if end point is approached slowly.)

(2) Transfer ca 0.3 g of  $\text{As}_2\text{O}_3$  (dried at  $110^\circ$ ) to 400 ml beaker. Add 10 ml of cool soln of  $\text{NaOH}$  (20%) and allow to stand until dissolved, stirring occasionally. Add 100 ml of  $\text{H}_2\text{O}$ , 10 ml of  $\text{HCl}$  (sp. gr. 1.18), and 1 drop of 0.0025 *M*  $\text{KIO}_3$  or KI. Titrate with the  $\text{KMnO}_4$  soln until faint pink color persists 30 seconds, adding last 1–1.5 ml dropwise and allowing each drop to become decolorized before adding the next. Determine by blank test with all reagents except  $\text{As}_2\text{O}_3$  the volume of  $\text{KMnO}_4$  (usually ca 0.03 ml) required to duplicate pink color of end point. (End point may also be taken with ferrous phenanthroline indicator, in which case 1 drop of 0.025 *M* soln of the indicator is added as end point is approached.) Determine blank correction. Titration can also be conducted potentiometrically.

(b) *Ferric sulfate*.—Dissolve 135 g of  $\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$  or 55 g of  $\text{Fe}_2(\text{SO}_4)_3$  (anhydrous) and dilute to 1 liter. Determine  $\text{Fe}_2(\text{SO}_4)_3$  in stock supply by strong ignition to  $\text{Fe}_2\text{O}_3$ . Titrate 50 ml of the  $\text{Fe}_2(\text{SO}_4)_3$  soln, acidified with 20 ml of 4 *N*  $\text{H}_2\text{SO}_4$ , with  $\text{KMnO}_4$ , and use this titer as zero-point correction.

(c) *Ferrous phenanthroline indicator*.—Dissolve 0.7425 g of orthophenanthroline monohydrate in 25 ml of 0.025 *M*  $\text{FeSO}_4$  soln (6.95 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  in 1 liter).

## 34.44

## DETERMINATION

Filter the  $\text{Cu}_2\text{O}$  in Gooch crucible and wash beaker and precipitate thoroly. Transfer asbestos film to beaker with aid of glass rod. Add 50 ml of the  $\text{Fe}_2(\text{SO}_4)_3$  soln and stir vigorously until  $\text{Cu}_2\text{O}$  is completely dissolved. Examine for complete solution, holding beaker above level of eye. Add 20 ml of 4 *N*  $\text{H}_2\text{SO}_4$  and titrate with standard  $\text{KMnO}_4$  soln. As end point is approached, add 1 drop of the ferrous phenanthroline indicator. At end point, brownish soln changes to green. Obtain weight of reducing sugar equivalent to weight of Cu from 44.12.

34.45 *By Electrolytic Deposition from Nitric Acid Solution (29)—Official*

Decant the hot soln thru asbestos mat in Gooch crucible and wash beaker and precipitate thoroly with hot  $\text{H}_2\text{O}$ . Transfer asbestos mat from crucible to beaker with glass rod and rinse crucible with 14 ml of  $\text{HNO}_3$  (1+1), allowing rinsings to flow into beaker. After the  $\text{Cu}_2\text{O}$  has dissolved, dilute to 100 ml, heat to boiling, and continue boiling ca 5 min. to remove oxides of N. Cool, filter, transfer to 250 ml beaker, and dilute to 200 ml. Add 1 drop of 0.1 *N*  $\text{HCl}$  and mix thoroly.

For electrolysis use cylindrical electrodes of Pt gauze, ca 1.5" and 2", respectively, in diam. and ca 1.75" in height, thoroly cleaned, ignited, cooled in desiccator, and weighed. Insert electrodes in Cu soln so that surface of cathode clears anode by at least 5 mm, and both electrodes almost touch bottom of beaker. Cover with split watch-glass to avoid loss by spattering. Electrolyze with current of 0.2–0.4 ampere until deposition is complete, usually overnight. (Wash down sides of beaker and watch-glass with  $\text{H}_2\text{O}$ , thus raising level of soln and exposing new surface of cathode; if new surface shows deposit of Cu, electrolysis is not complete.) Without interrupting current, slowly lower beaker and at same time wash electrodes with stream of  $\text{H}_2\text{O}$ . Immediately immerse electrodes in another beaker of  $\text{H}_2\text{O}$  and break current. (Washing may also be accomplished by use of siphon,  $\text{H}_2\text{O}$  being added as soln is removed; displacement of  $\text{HNO}_3$  soln is complete when current ceases to flow.) Rinse cathode with ethyl alcohol and dry for a few minutes in oven at 100°. Cool in desiccator and weigh.

Electrolyte may be stirred by rotating anode or mechanical stirrer. In this case current may be increased to 1–2 amperes, thus shortening time required for complete deposition of Cu to ca 1 hour.

If extreme care is exercised to avoid spattering, the  $\text{Cu}_2\text{O}$  can be dissolved by allowing the  $\text{HNO}_3$  to flow down walls of crucible. Keep crucible covered as much as possible with small watch-glass. Collect filtrate in beaker and wash watch-glass and tip of pipet with jet of  $\text{H}_2\text{O}$ . Continue as directed above, beginning "dilute to 100 ml . . ."

## 34.46

## IV. Hertzfeld Gravimetric Method—Official

(Materials containing 1.5% or less of invert sugar and 98.5% or more of sucrose)

Prepare soln of material to be examined containing 20 g in 100 ml, clarify with

neutral Pb acetate, and remove excess Pb with  $\text{Na}_2\text{C}_2\text{O}_4$ . Place 50 ml of reagent, 34.33, and 50 ml of the sugar soln in 250 ml beaker. Heat this mixture at such a rate that ca 4 min. is required to bring it to boiling point and boil for exactly 2 min. Add 100 ml of cold recently boiled  $\text{H}_2\text{O}$ . Filter immediately thru asbestos, 34.38, and determine Cu by one of methods described under 34.41–34.45. Obtain corresponding percentage of invert sugar from 44.12.

#### V. Ofner Volumetric Method—Tentative

(Materials containing small quantities of invert sugar in presence of sucrose)

##### 34.47

##### REAGENTS

(a) *Copper soln.*—Dissolve 5.0 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 10.0 g of anhydrous  $\text{Na}_2\text{CO}_3$ , 300 g of pulverized Rochelle salt, and 50 g of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  (or 19.8 g of the anhydrous salt) in ca 900 ml of  $\text{H}_2\text{O}$ , warming finally if necessary. When completely dissolved it is advisable to continue heating for 2 hours on water bath to destroy mold spores. Cool, and adjust volume to 1 liter. Treat with active C or kieselgühr and filter, or filter directly thru sintered glass having fine pores. Determine Cu content by electrolysis and so adjust that soln will contain 63.4 mg of Cu in 50 ml. Preserve in dark place.

(b) *Sodium thiosulfate soln.*—Dissolve 4.00 g of crystals and dilute to volume of 500 ml or, preferably, prepare stock soln containing in 500 ml, 20.0 g of crystals and 1 ml of *N* NaOH or 0.1 g of  $\text{Na}_2\text{CO}_3$ . Dilute 100 ml of this stock soln to 500 ml as required. Standardize by titration against the I soln (c).

(c) *Iodine soln.*—Dissolve 2.05 g of pure I in ca 10 g of iodate-free KI dissolved in a few ml of  $\text{H}_2\text{O}$ . Dilute to volume of exactly 500 ml and preserve in dark place. This soln is 0.03230 *N*.

(d) *Starch soln.*—Rub 2.5 g of soluble starch and ca 10 mg of  $\text{HgI}_2$  in a little  $\text{H}_2\text{O}$ . Dissolve in ca 500 ml of boiling  $\text{H}_2\text{O}$ .

##### 34.48

##### DETERMINATION

Dissolve 20 g of sample in  $\text{H}_2\text{O}$  and dilute to 100 ml. Transfer 50 ml of soln containing not more than 20 mg of invert sugar to 300 ml Erlenmeyer flask and add 50 ml of the Cu reagent. Mix well, add 50–100 mg of pumice or talcum powder, and bring to boiling on asbestos gauze plate in course of 4–5 min. Judge initial time of boiling, not when bubbles arise from bottom of flask, but when they burst at surface in considerable number. Continue boiling for exactly 5 min. Cool without agitation by immersion in cold  $\text{H}_2\text{O}$ . Add 1 ml of acetic acid. Add with continuous agitation an accurately measured volume of I soln, 5–30 ml according to amount of Cu reduced, being sure that excess is finally present. Pour down wall of flask from graduated cylinder 15 ml of normal HCl. Stopper flask and allow I to react for ca 2 min., occasionally agitating soln. Titrate excess of I with the  $\text{Na}_2\text{S}_2\text{O}_3$  soln, adding starch as end point is approached. Deduct volume of excess I from volume added. Obtain amount of invert sugar present by applying proper correction to volume of I soln consumed, 44.14. After applying correction, each ml of 0.0323 *N* I consumed corresponds to 1 mg of invert sugar.

##### 34.49

#### VI. Meissl-Hiller Gravimetric Method—Official

(Materials containing more than 1.5% of invert sugar and less than 98.5% of sucrose)

Prepare soln of suitable concentration of material to be examined, clarify with neutral Pb acetate, and remove excess of Pb. Prepare series of solns in large test tubes by adding 1, 2, 3, 4, and 5 ml of this soln to each tube successively. Add 5 ml of the reagent, 34.33, to each, heat to boiling, boil 2 min., and filter. Note volume of sugar soln that gives the filtrate lightest in tint, but still distinctly blue. Place 20 times this volume of the sugar soln in 100 ml flask, dilute to mark, and mix well. Use 50 ml of diluted soln for the determination, and proceed as directed under 34.46. For calculation of result use following formulas and table of factors of Meissl and Hiller, 44.15.

Let  $Cu$  = weight of Cu obtained;  $P$  = polarization of sample;  $W$  = weight of sample in 50 ml of the soln used for determination; and  $F$  = factor obtained from table for conversion of Cu to invert sugar.

Then  $\frac{Cu}{2} = Z$ , approx. weight of invert sugar;  $Z \times \frac{100}{W} = Y$ , approx. percentage of invert sugar;  $\frac{100 P}{P + Y} = R$ , approx. percentage of sucrose in mixture of sugars;  $100 - R = I$ , approx. percentage of invert sugar; and  $\frac{CuF}{W}$  = percentage of invert sugar.

Use factor  $F$  for calculating Cu to invert sugar, 44.15. *Example:* Polarization of a sugar is 86.4, and 50 ml of soln containing 3.256 g of sample gives 0.290 g of Cu.

$$\frac{Cu}{2} = \frac{0.290}{2} = 0.145 = Z. \quad \frac{Z \times 100}{W} = 0.145 \times \frac{100}{3.256} = 4.45 = Y.$$

$$\frac{100 P}{P + Y} = \frac{86.40}{86.4 + 4.45} = 95.1 = R. \quad 100 - R = 100 - 95.1 = I = 4.9. \quad R:I = 95.1:4.9.$$

Table shows that vertical column headed 150 is nearest to  $Z$ , 145, and horizontal column headed 95.5 is nearest to ratio of  $R$  to  $I$ , 95.1:4.9. Where these columns meet is found the factor 51.2, which enters into final calculation:

$$\frac{CuF}{W} = \frac{0.290 \times 51.2}{3.256} = 4.56\% \text{ of invert sugar.}$$

#### DEXTROSE—CHEMICAL METHODS

##### 34.50 *Lane-Eynon General Volumetric Method—Tentative*

Proceed as directed under 34.35, referring titer to 44.19 or 44.20.

##### 34.51 *Munson-Walker General Method—Official*

Proceed as directed under 34.39 and obtain from 44.11 or 44.12, weight of dextrose

##### *Allihn Gravimetric Method—Official*

##### 34.52

##### REAGENTS

(a) *Copper sulfate soln.*—Dissolve 34.639 g of  $CuSO_4 \cdot 5H_2O$  in  $H_2O$  and dilute to 500 ml.

(b) *Alkaline tartrate soln.*—Dissolve 173 g of Rochelle salt and 125 g of KOH in  $H_2O$  and dilute to 500 ml.

## 34.53

## DETERMINATION

Place 30 ml of the  $\text{CuSO}_4$  soln, 30 ml of the alkaline tartrate soln, and 60 ml of  $\text{H}_2\text{O}$  in beaker, and heat to boiling. Add 25 ml of soln of material to be examined containing not more than 0.25 g of dextrose, and boil exactly 2 min., keeping beaker covered. Filter immediately thru asbestos and obtain weight of Cu by one of methods given under 34.41–34.45. Obtain corresponding weight of dextrose from 44.18.

## LEVULOSE—CHEMICAL METHODS

## 34.54

## I. Lane-Eynon General Volumetric Method—Official ✓

Proceed as directed under 34.35, referring titer to 44.19 or 44.20.

## 34.55

## II. Munson-Walker General Method—Official

Proceed as directed under 34.39 and 34.45, and obtain from 44.12, weight of levulose equivalent to weight of Cu.

## III. Jackson-Mathews Modification of Nyns Selective Method (30)—Official

## 34.56

## REAGENT

*Ost soln.*—Dissolve 250 g of  $\text{K}_2\text{CO}_3$  (anhydrous) in ca 700 ml of hot  $\text{H}_2\text{O}$ , add 100 g of pulverized  $\text{KHCO}_3$ , and agitate mixture until completely dissolved. Cool, and add with very vigorous agitation a soln of 25.3 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 100–150 ml of  $\text{H}_2\text{O}$ . Make to 1 liter and filter.

## 34.57

## DETERMINATION

Transfer 50 ml of the Ost soln to 150 ml Erlenmeyer flask and add, by means of accurately graduated pipet, volume of soln to be analyzed that contains not more than 92 mg of levulose or its equivalent of a levulose-dextrose mixture, remembering that dextrose has about one-twelfth the reducing power of levulose. Add enough  $\text{H}_2\text{O}$  to make total volume 70 ml. Immerse in water bath, regulated preferably within  $0.1^\circ$ , at  $55^\circ$ . Digest for exactly 75 min., agitating with rotary motion at intervals of 10 or 15 min.

Filter the precipitated  $\text{Cu}_2\text{O}$  on closely packed Gooch crucible and wash flask and precipitate thoroly without attempting to transfer precipitate quantitatively. Determine Cu by one of methods described under 34.41–34.45. As it is usually difficult to transfer the Cu precipitate quantitatively from the Erlenmeyer flask, select a method of Cu analysis in which total Cu is dissolved in  $\text{HNO}_3$  and determined by electrolysis or  $\text{Na}_2\text{S}_2\text{O}_3$  titration, or in a  $\text{Fe}_2(\text{SO}_4)_3$  soln followed by  $\text{KMnO}_4$  titration as directed in 34.44.

See 44.17 for levulose equivalent. If sample contained dextrose in addition to levulose, analytical result is not true but "apparent" levulose, as dextrose has appreciable reducing action under conditions of analysis. To determine correction for dextrose analyze the sample also for total reducing sugar and compute true dextrose and levulose by series of approximations. Calculate percentage of reducing sugar in original sample and similarly percentage of "apparent" levulose. Difference between these two percentages is the "apparent" dextrose. Divide the apparent dextrose by factor 12.4 and deduct result from apparent levulose to obtain a new approximation to the true levulose. Deduct the new levulose percentage from total reducing sugar percentage to obtain a more correct value for true dextrose and

again divide by 12.4. Deduct quotient from original value of the "apparent" levulose and continue approximation in same manner until percentage of levulose remains essentially unaltered by two successive approximations.

If original sample contained sucrose, determine by means of Clerget procedure, 34.23. Correct Cu for reducing action of sucrose before referring to the table, 44.17. 1, 2, 3, 4, and 5 g of sucrose precipitate under the conditions of the analysis 3.3, 5.7, 7.4, 8.5, 9.0 mg of Cu, respectively.

#### MALTOSE—CHEMICAL METHODS

##### 34.58 *Lane-Eynon General Volumetric Method—Official*

Proceed as directed under 34.35, referring titer to 44.19 or 44.20.

##### 34.59 *Munson-Walker General Method—Official*

Proceed as directed under 34.39 and 34.40 and obtain from 44.11 weight of maltose equivalent to weight of  $\text{Cu}_2\text{O}$ .

#### LACTOSE—CHEMICAL METHODS

##### 34.60 *Lane-Eynon General Volumetric Method—Official*

Proceed as directed under 34.35, referring titer to 44.19 or 44.20.

##### 34.61 *Munson-Walker General Method—Official*

Proceed as directed under 34.39 and 34.40 and obtain from 44.11, weight of lactose equivalent to weight of  $\text{Cu}_2\text{O}$ .

#### 34.62 REDUCING SUGARS OTHER THAN DEXTROSE—OFFICIAL

Proceed as directed under 34.53. Multiply weight of dextrose found by following factors: arabinose, 0.969; xylose, 1.017; and galactose, 1.114.

#### MICRO METHOD FOR REDUCING SUGARS (51)—TENTATIVE

##### 34.63

##### REAGENT

Dissolve 12 g of Rochelle salt, 20 g of  $\text{Na}_2\text{CO}_3$ , and 25 g of  $\text{NaHCO}_3$  in ca 500 ml of  $\text{H}_2\text{O}$  and into this soln pour with stirring 6.5 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  dissolved in ca 100 ml of  $\text{H}_2\text{O}$ ; add a soln composed of 10 g of KI, 0.80 g of  $\text{KIO}_3$ , and 18 g of  $\text{K}_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$ , and dilute to 1 liter. Only the  $\text{KIO}_3$  need be weighed accurately.

##### 34.64

##### DETERMINATION

Measure 5 ml (accurately) of reagent into test tube (25×250 mm) and add 5 ml of the sugar soln containing not less than 0.1 mg nor more than 2 mg of dextrose. Mix by gentle shaking, cover tube, and keep in boiling water bath 15 min. Cool in water bath to 35–40°. Add with agitation 1 ml of 5 N  $\text{H}_2\text{SO}_4$  and see that all  $\text{Cu}_2\text{O}$  is dissolved. After ca 2 min. titrate with 0.005 N  $\text{Na}_2\text{S}_2\text{O}_4$  soln, using starch indicator. Determine blank titration on 5 ml of reagent after heating with 5 ml of  $\text{H}_2\text{O}$ . Deduct titer of determination from that of blank and refer result to 34.65.

NOTE: It is important to run control analyses with pure dextrose and apply a correction for any deviation from tabulated equivalents.

## 34.65

*Somogyi\* Dextrose-Thiosulfate Equivalents*

(Quantities of dextrose corresponding to titration values when 5 ml of soln and 5 ml of Cu reagent are heated in water bath for 15 min.)

0.005 N THIO- SULFATE (ML)	TENTHS OF 1 ML OF 0.005 N SODIUM THIOSULFATE									
	0	1	2	3	4	5	6	7	8	9
	DEXTROSE IN 5 ML SOLUTION (MG)									
0			.11	.12	.13	.15	.16	.17	.18	.20
1	.21	.22	.23	.25	.26	.27	.28	.29	.31	.32
2	.33	.34	.35	.36	.38	.39	.40	.41	.42	.43
3	.45	.46	.47	.485	.495	.505	.515	.530	.540	.550
4	.565	.575	.585	.595	.605	.620	.630	.640	.650	.660
5	.670	.685	.695	.705	.715	.730	.740	.750	.760	.770
6	.785	.795	.805	.815	.825	.840	.850	.860	.870	.880
7	.895	.905	.915	.925	.935	.950	.960	.970	.980	.995
8	1.005	1.015	1.025	1.035	1.050	1.060	1.070	1.080	1.090	1.105
9	1.115	1.125	1.135	1.150	1.160	1.170	1.185	1.195	1.205	1.215
10	1.225	1.240	1.250	1.260	1.270	1.280	1.295	1.305	1.315	1.325
11	1.335	1.350	1.360	1.370	1.380	1.395	1.405	1.415	1.425	1.440
12	1.450	1.460	1.470	1.480	1.495	1.505	1.515	1.525	1.540	1.550
13	1.560	1.570	1.580	1.590	1.605	1.615	1.630	1.640	1.650	1.660
14	1.670	1.685	1.695	1.705	1.715	1.725	1.735	1.750	1.760	1.770
15	1.780	1.795	1.805	1.815	1.825	1.835	1.850	1.860	1.870	1.880
16	1.890	1.905	1.915	1.930	1.940	1.950	1.960	1.970	1.980	1.990
17	2.000									

\* M. Somogyi, *J. Biol. Chem.*, **70**, 607 (1926). 1 ml of 0.005 N thiosulfate = 0.318 mg of Cu.

## CONFECTIONERY

## 34.66

## PREPARATION OF SAMPLE—OFFICIAL

If composition of entire sample is desired, grind and mix thoroly. If sample is composed of layers or of distinctly different portions and it is desired to examine these individually, separate with knife or other mechanical means as completely as possible and grind and mix each portion thoroly.

34.67 MOISTURE—OFFICIAL.—See 34.2, 34.3, 34.4, or 34.5

34.68 ASH—OFFICIAL.—See 34.9 or 34.10

34.69 MINERAL CONSTITUENTS—OFFICIAL.—See Chap. 12

34.70 SOLUBLE AND INSOLUBLE ASH—OFFICIAL.—See 34.13

34.71 ALKALINITY OF SOLUBLE ASH—OFFICIAL.—See 34.14

34.72 ALKALINITY OF INSOLUBLE ASH—OFFICIAL.—See 34.15

34.73 MINERAL ADULTERANTS IN ASH—TENTATIVE.—See 34.16

34.74 NITROGEN—OFFICIAL

Determine N in 2–5 g of the material as directed under 2.24, 2.25, or 2.26, using a larger quantity of  $H_2SO_4$  if necessary for complete digestion.

## SUCROSE—POLARIMETRIC METHODS

34.75 *In Absence of Raffinose—Official.*—See 34.23, 34.24, or 34.29



## SUCROSE—CHEMICAL METHODS

**34.76** *By Reducing Sugars Before and After Inversion—Official.—See 34.30*

**34.77** **COMMERCIAL GLUCOSE—OFFICIAL.—See 34.31 or 34.32**

**34.78** **STARCH—TENTATIVE**

Measure 25 ml of soln of uniform mixture (representing 5 g of sample) into 300 ml beaker, or introduce into beaker 5 g of finely ground sample (previously extracted with ether if sample contains much fat); add sufficient  $H_2O$  to make volume 100 ml; heat to ca  $60^\circ$  (avoiding if possible gelatinizing the starch); and allow to stand ca 1 hour, stirring frequently to secure complete soln of sugars. Transfer to wide-mouthed bottle, rinse beaker with a little warm  $H_2O$ , and cool. Add equal volume of alcohol, mix, and allow to stand at least 1 hour. Centrifuge until precipitate is closely packed on bottom of bottle and decant supernatant liquid thru hardened filter. Wash precipitate with successive 50 ml portions of alcohol, 50% by volume, by centrifuging and decanting thru filter until washings give no test for sugar when tested as follows: Introduce into test tube few drops of the washings, 3 or 4 drops of 20% alcoholic  $\alpha$ -naphthol soln and 2 ml of  $H_2O$ . Shake well, tip tube, allow 2–5 ml of  $H_2SO_4$  to flow down side of tube, then hold tube upright. If sugar is present interface of two liquids will be colored faint to deep violet; on shaking, whole soln will become blue violet. Transfer residue from bottle and hardened filter to large flask and determine starch as directed under 27.35.

## ETHER EXTRACT—TENTATIVE

**34.79** *I. Continuous Extraction Method.*—Measure 25 ml of a 20% mixture or soln into very thin, readily frangible glass evaporating shell, or thin Pb or Sn foil dish containing 5–7 g of freshly ignited asbestos fiber; or, if possible to obtain uniform sample, weigh 5 g of mixed finely divided sample into dish and wash with  $H_2O$  upon the asbestos in the evaporating shell using, if necessary, small portion of the asbestos fiber on stirring rod to transfer last traces of sample from dish to shell. Dry to constant weight at  $100^\circ$ , cool, wrap glass dish loosely in smooth paper, crush into rather small fragments between fingers, and carefully transfer crushed mass, including paper, to extraction tube or fat extraction cartridge. If metal dish is used, cut into small pieces and place in extraction tube. Extract with anhydrous ether or petroleum benzine (b.p.  $45$ – $60^\circ$  and without weighable residue) in continuous extraction apparatus for at least 25 hours. In most cases it is advisable to remove the substance from extractor after first 12 hours, grind with sand to fine powder, and re-extract for remaining 13 hours. Transfer extract to weighed flask, evaporate solvent, and dry to constant weight at  $100^\circ$ .

**34.80** *II. Roese-Gottlieb Method.*—Introduce 4 g of material, or quantity of a uniform soln equivalent to this weight of the dry substance, into Röhrig tube or similar apparatus; make up to volume of 10 ml with  $H_2O$ , add 1.25 ml of  $NH_4OH$ , and mix thoroly. Add 10 ml of alcohol and mix; then add 25 ml of ether and shake vigorously for half a minute; and finally add 25 ml of petroleum benzine (b.p. below  $60^\circ$ ) and shake again for half a minute. Allow to stand for 20 min. or until separation of liquids is complete. Draw off as much as possible of the benzine-fat soln (usually 0.5–0.8 ml will be left) into weighed flask thru small, rapid filter. Weigh flask with similar one as counterpoise. Again extract liquid remaining in tube, this time with 15 ml each of ether and petroleum benzine; shake vigorously half a minute with each solvent and allow to settle. Proceed as above, washing tip of spigot and filter with a few ml of a mixture of equal parts of the 2 solvents (previously mixed and freed from deposited  $H_2O$ ). For greater degree of accuracy, extraction must be repeated.

If previous solvent-fat solns have been drawn off closely, this third extraction usually yields not more than ca 1 mg of fat, or ca 0.02% on 4 g charge. Evaporate solvent slowly on steam bath and then dry fat in boiling water oven until loss in weight ceases. Test purity of the fat by dissolving in a little petroleum benzine. Should a residue remain, wash fat out completely with petroleum benzine, dry residue, weigh, and deduct weight.

34.81

## PARAFFIN—TENTATIVE

Add to solvent extract in flask, 34.79 or 34.80, 10 ml of alcohol and 2 ml of NaOH soln (1+1); connect flask with reflux condenser; and heat for 1 hour on water bath, or until saponification is complete. Remove condenser and allow flask to remain on bath until alcohol is evaporated and residue is dry. Dissolve residue as completely as possible in ca 40 ml of H<sub>2</sub>O and heat on bath, shaking frequently. Wash into separator, cool, and extract with 4 successive portions of petroleum benzine, collecting extracts in weighed flask or capsule. Evaporate petroleum benzine and dry to constant weight at 100°. Any phytosterol or cholesterol present in fat would be extracted with the paraffin, but quantity is so insignificant that it may generally be disregarded.

34.82

ALCOHOL IN SIRUPS USED IN CONFECTIONERY  
("BRANDY DROPS")—OFFICIAL

Collect in beaker the sirup from sufficient number of pieces to yield 30–50 g, strain into weighed beaker, and weigh. Introduce the sirup into 250–300 ml distilling flask, dilute with half its volume of H<sub>2</sub>O, attach flask to vertical condenser, and distil almost 50 ml, or as much of liquid as possible without causing charring. Foaming may be prevented by adding to contents of distillation flask a little tannin, or piece of paraffin about size of a pea. Cool distillate, make up to volume with H<sub>2</sub>O, and mix well. Determine sp. gr. as directed under 16.4. Calculate percentage of alcohol by weight in candy filling, using table 44.23.

34.83

## COLORING MATTERS—TENTATIVE.—See Chap. 21

34.84

## METALS—TENTATIVE.—See Chap. 29

## HONEY (32)

34.85

## PREPARATION OF SAMPLE—OFFICIAL

(a) *Liquid or strained honey*.—If sample is free from granulation, mix thoroly by stirring or shaking before weighing portions for determinations. If honey is granulated, place container, having stopper loose, in water bath and heat at temp. not exceeding 50° with occasional stirring until sugar crystals dissolve. Mix thoroly, cool, and weigh portions for determinations. If foreign matter, such as wax, sticks, bees, particles of comb, etc., is present, heat sample to 40° in water bath and strain thru cheese-cloth in hot water funnel before weighing portions for analysis.

(b) *Comb honey*.—Cut across top of comb, if sealed, and separate completely from comb by straining thru 40-mesh sieve. When portions of comb or wax pass thru sieve, heat sample as directed in (a) and strain thru cloth. If honey is granulated in comb, heat until wax is liquefied; stir, cool, and remove wax.

## MOISTURE

34.86

## Direct Drying—Official

Proceed as directed under 34.4 or 34.5, using a weighed quantity of sample sufficient to yield ca 1 g of solids; adding, if necessary, a few ml of H<sub>2</sub>O to incorporate

thoroly with the sand; and drying at 70° under pressure of not to exceed 50 mm of Hg.

**34.87***By Means of Refractometer—Official*

Determine refractometer reading of soln at 20° and obtain corresponding percentages of moisture from 34.88. If determination is made at temp. other than 20°, correct reading to standard temp. of 20° according to footnote.

**34.88** *Chataway table showing relationship between refractive index and moisture content and weight per gallon of honey*

REFRACTIVE INDEX AT 20°	MOISTURE	WT. OF HONEY IN POUNDS/GALLON AT 20°		REFRACTIVE INDEX AT 20°	MOISTURE	WT. OF HONEY IN POUNDS/GALLON AT 20°	
	per cent	lb.	oz.		per cent	lb.	oz.
1.5041	13.0			1.4935	17.2		
35	.2	12	1	30	.4	11	13
30	.4			25	.6		
25	.6			20	.8		
20	.8	12	½	15	18.0	11	12½
15	14.0			10	.2		
10	.2	12	0	05	.4		
05	.4			00	.6	11	12
00	.6			1.4895	.8		
1.4995	.8	11	15½	90	19.0	11	11½
90	15.0			85	.2		
85	.2			80	.4		
80	.4	11	15	76	.6	11	11
75	.6			71	.8		
70	.8	11	14½	66	20.0		
65	16.0			62	.2	11	10½
60	.2			58	.4		
55	.4	11	14	53	.6	11	10
50	.6			49	.8		
45	.8			1.4844	21.0		
40	17.0	11	13½				

Temperature corrections: Refractive Index, 0.00023/°C, or 0.00013/°F; pounds per gallon, ½ oz. per 6°C or 11°F. If the reading is made at temp. above 20° C (68° F) add the correction; if made below, subtract the correction. *Can. J. Research*, 6, 532 (1932); 8, 435 (1933); *Canadian Bee J.*, August 1935, p. 215; *J. Assoc. Official Agr. Chem.*, 25, 99, 681 (1942).

**34.89****ASH—OFFICIAL**

Weigh 5–10 g of honey into Pt dish, add a few drops of pure olive oil to prevent spattering, heat carefully until swelling ceases, and ignite at temp. not above dull redness (ca 600°) until white ash is obtained.

**34.90****SOLUBLE ASH—OFFICIAL.—See 34.13****34.91****ALKALINITY OF SOLUBLE ASH—OFFICIAL.—See 34.14****34.92****DIRECT POLARIZATION—TENTATIVE**

(a) *Immediate direct polarization.*—Transfer 26 g of honey to 100 ml flask with H<sub>2</sub>O, add 5 ml of alumina cream, dilute to mark with H<sub>2</sub>O at 20°, filter, and polarize immediately in 200 mm tube.

(b) *Constant direct polarization.*—Complete mutarotation as directed under 34.21. If necessary to conserve sample, soln from tube used in immediate direct polarization (a) may be returned to flask. Make final reading at 20° in 200 mm tube.

(c) *Mutarotation.*—Difference between (a) and (b) is measure of the mutarotation.

(d) *Direct polarization at 87°*.—Polarize soln obtained under (b) at 87° in a jacketed 200 mm metal tube.

**34.93****INVERT POLARIZATION—TENTATIVE**

(a) *At 20°*.—Invert 50 ml of soln, 34.92, as directed under 34.23(b) or (c) or 34.24(b) or (c), and polarize at 20° in 200 mm tube.

(b) *At 87°*.—Polarize soln (a) at 87° in 200 mm metal tube.

**34.94****REDUCING SUGARS—OFFICIAL**

Dilute 10 ml of soln, 34.92, to 250 ml and determine reducing sugars in 25 ml of this soln by one of methods given, 34.35 or 34.39. Calculate result to percentage of invert sugar.

**34.95****SUCROSE—OFFICIAL**

(a) Calculate from data given in 34.92(b) and 34.93(a) if inversion is conducted as directed under 34.23(b) or (c). Use formula given in 34.23(b).

(b) Proceed as directed under 34.30. Determine reducing sugars after inversion by diluting 10 ml of soln, 34.93, with small quantity of H<sub>2</sub>O, neutralizing with Na<sub>2</sub>CO<sub>3</sub>, and making up to 250 ml with H<sub>2</sub>O. Use 50 ml of this soln, making determination as directed in 34.94.

**34.96****LEVULOSE—TENTATIVE**

Multiply direct reading at 87°, 34.92(d), by 1.0315 and subtract product from constant direct polarization at 20°, 34.92(b); divide difference by 2.3919 to obtain grams of levulose in normal weight of honey. From this figure calculate percentage of levulose in original sample. Or determine levulose selectively by 34.57.

**34.97****DEXTROSE—TENTATIVE**

To obtain approximate percentage of dextrose, subtract percentage of levulose, 34.96, from percentage of invert sugar, 34.94.

The dextrose can be determined more accurately by multiplying percentage of levulose, 34.96, by factor 0.915, which gives its dextrose equivalent in Cu reducing power. Subtract figure obtained from that of reducing sugars, 34.94, calculated as dextrose, to obtain percentage of dextrose in sample. Owing to difference in reducing powers of different sugars, sum of dextrose thus found and the levulose, 34.96, will be greater than quantity of invert sugar obtained under 34.94.

**34.98****DEXTRIN (APPROXIMATE)—TENTATIVE**

Using not more than 4 ml of H<sub>2</sub>O, transfer 8 g of sample (4 g in case of dark colored honeydew honey) to 100 ml flask by allowing sample to drain from weighing dish into flask and then dissolving residue in 2 ml of H<sub>2</sub>O. After adding this soln to contents of flask, rinse weighing dish with two 1 ml portions of H<sub>2</sub>O, adding a few ml of absolute alcohol each time before decanting. Fill flask to mark with absolute alcohol, shaking constantly. Set flask aside until dextrin has collected on sides and bottom and liquid is clear. Decant the clear liquid thru filter paper and wash residue in flask with 10 ml of alcohol, pouring washings thru same filter. Dissolve dextrin in flask with boiling H<sub>2</sub>O and filter thru the filter paper already used, receiving filtrate in weighed dish prepared as directed under 34.5. Rinse flask and wash filter a number of times with small portions of hot H<sub>2</sub>O, evaporate on water bath, and dry to constant weight at 70° under pressure not exceeding 50 mm of Hg.

After determining weight of alcohol precipitate, dissolve latter in  $H_2O$  and make up to definite volume, using 50 ml of  $H_2O$  for each 0.5 g of precipitate or part thereof.

Determine reducing sugars in soln both before and after inversion as directed under 34.30, expressing results as invert sugar. Calculate sucrose from results thus obtained and subtract sum of reducing sugars before inversion and sucrose from weight of total alcohol precipitate to obtain weight of dextrin.

## 34.99

## FREE ACID—OFFICIAL

Dissolve 10 g of honey in  $H_2O$  and titrate with 0.1 *N* NaOH, using phenolphthalein indicator. Express results in terms of ml of 0.1 *N* NaOH required to neutralize 100 g of sample.

## COMMERCIAL GLUCOSE

## 34.100

*Qualitative Test—Tentative*

Dilute the honey with  $H_2O$  in proportion of 1 to 1 and add a few ml of I soln (1 g of I, 3 g of KI, 50 ml of  $H_2O$ ). In presence of commercial glucose the soln turns red or violet, depth and character of color depending upon quality and nature of glucose used. Blank test with a pure honey of about same color should be made in order to secure accurate color comparison. Should the honey be dark and the percentage of glucose very small, precipitate the dextrin that may be present by adding several volumes of alcohol. Allow to stand until precipitate settles (do not filter), decant liquid, dissolve residue of dextrins in hot  $H_2O$ , cool, and apply above test to this soln. Negative result is not proof of absence of commercial glucose, as some glucose, especially of high conversion, does not give any reaction with I (33).

## 34.101

*Quantitative Method—Tentative*

An approximate determination can be made by Browne's formula as follows: Multiply difference in polarizations of invert soln at 20° and 87°, 34.93, by 77 and divide this product by percentage of invert sugar found in sample after inversion. Multiply quotient by 100 and divide product by 26.7 to obtain percentage of honey in sample; 100% minus percentage of honey gives percentage of glucose (33).

## COMMERCIAL INVERT SUGAR (34)

*Resorcinol Test (35)—Tentative*

## 34.102

## REAGENT

*Resorcinol soln.*—Dissolve 1 g of resublimed resorcinol in 100 ml of HCl (sp. gr. 1.18–1.19).

## 34.103

## DETERMINATION

Introduce 10 ml of a 50% honey soln into test tube and add 5 ml of ether. Shake gently and allow to stand until ether layer is clear. Transfer 2 ml of this clear ether soln to small test tube and add a large drop of the recently prepared resorcinol soln. Shake, and note color. Cherry red color appearing within 1 min. indicates presence of commercial invert sugar. Yellow to salmon shades have no significance.

*Aniline Chloride Test (36)—Tentative*

## 34.104

## REAGENT

*Aniline chloride soln.*—To 100 ml of C.P. aniline add 30 ml of 25% HCl.

## 34.105

## DETERMINATION

Introduce 5 g of the honey into porcelain dish and add while stirring 2.5 ml of the recently prepared aniline reagent. In presence of commercial invert sugar, reagent assumes within 1 min. an orange-red color turning dark red. Yellow to salmon shades have no significance.

Resorcinol test and aniline chloride test, when negative, may not be regarded as conclusive evidence of absence of commercial invert sugar sirup in honey.

## 34.106

## DIASTASE (37)—TENTATIVE

Mix 1 part of honey with 2 parts of sterile  $H_2O$ . Treat 10 ml of this soln with 1 ml of 1% soluble starch soln and digest at  $45^\circ$  for 1 hour. Test mixture with 1 ml of I soln (1 g of I, 2 g of KI, 300 ml of  $H_2O$ ). Treat another 10 ml portion of the honey soln, mixed with 1 ml of the soluble starch soln without heating to  $45^\circ$ , with the reagent and compare colors produced. If original honey has not been heated sufficiently to destroy the diastase, an olive-green or brown coloration will be produced in mixture that has been heated at  $45^\circ$ . Heated or artificial honey becomes blue.

## MAPLE PRODUCTS (38)

## 34.107

## PREPARATION OF SAMPLE

(a) *Maple Sirup—Official*

(1) *For solids determination.*—If sample contains no sugar crystals or suspended matter, decant sufficient of clear sirup for use in determination. If sugar crystals are present, redissolve by heating at ca  $50^\circ$ . If suspended matter is present, filter sample thru cotton wool.

(2) *For other determinations.*—If sugar crystals are present, redissolve by heating. If other sediment is present, distribute it evenly thru sirup by shaking. Transfer ca 100 ml of sirup, with its suspended sediment, to casserole or beaker, add  $\frac{1}{2}$  volume of  $H_2O$ , and evaporate over flame. When temp. of boiling sirup approaches  $104^\circ$ , draw small quantity into thin-walled pipet of ca 1 ml capacity, and cool to room temp. in running  $H_2O$ . Wipe outside of pipet, allow the possibly diluted sirup in the point to escape, transfer some of remaining sirup to refractometer, and determine solids content of the cooled sirup. Repeat this procedure from time to time until reading is obtained corresponding to 64.5% solids ( $n_{20} = 1.4521$ ), or to such other value as in experience of analyst will give filtered sirup of 65.0% solids. Filter sirup thru filter that will allow the 100 ml to pass within 5 min. and adjust filtrate to  $65.0 \pm 0.5\%$  solids (refractometric) by thoro mixing with appropriate quantity of  $H_2O$ .

(b) *Maple Sugar and Other Solid or Semi-Solid Products—Tentative*

(1) *For moisture and solids determination.*—Grind in mortar, if necessary, and mix thoroly.

(2) *For other determinations.*—Prepare a sirup by dissolving ca 100 g of sample in 150 ml of hot  $H_2O$ , boil until temp. approaches  $104^\circ$ , and complete preparation of resulting sirup as directed in (a) (2), commencing "draw small quantity into thin-walled pipet."

## MOISTURE OR SOLIDS—OFFICIAL

## 34.108

*Maple Sugar*

Proceed as directed under 34.2, or preferably 34.3, using sample prepared as directed under 34.1.

**34.109** *Maple Sirup, Maple Cream, etc.*

Proceed as directed under 34.3, 34.4, or 34.8, using sample prepared as directed under 34.107.

**34.110** **ASH—OFFICIAL**

Using 5 g of prepared sirup, 34.107(a)(2) or (b)(2), proceed as directed under 34.9 or 34.10.

**34.111** **SOLUBLE AND INSOLUBLE ASH—OFFICIAL.—See 34.13****34.112** **ALKALINITY OF SOLUBLE ASH—OFFICIAL.—See 34.14****34.113** **ALKALINITY OF INSOLUBLE ASH—OFFICIAL.—See 34.15****34.114** **ALKALINITY OF TOTAL ASH—OFFICIAL**

Add the alkalinities of the soluble and insoluble portions, 34.112 and 34.113.

**34.115** **METALS—TENTATIVE.—See Chap. 29****POLARIZATION—OFFICIAL****34.116** *Direct Polarization.—See 34.23(a)***34.117** *Invert Polarization*

(a) *At 20°.*—Proceed as directed under 34.23(b) or (c) or 34.24(b) or (c).

(b) *At 87°.*—Proceed as directed under 34.32.

**34.118** **SUCROSE—POLARIMETRIC METHODS—OFFICIAL**

Proceed as directed under 34.23 or 34.24, or calculate from results of 34.116 and 34.117, using appropriate formula from 34.23 or 34.24.

**SUCROSE—CHEMICAL METHODS****34.119** *By Reducing Sugars Before and After Inversion—Official.—See 34.30***34.120** **REDUCING SUGARS AS INVERT SUGAR—OFFICIAL**

(a) *Before inversion.*—Proceed as directed under 34.35 or 34.39, using aliquot of soln used for direct polarization, 34.116. If soln is clarified, only neutral Pb acetate soln may be used, and excess of Pb must be removed with dry  $\text{Na}_2\text{C}_2\text{O}_4$ .

(b) *After inversion.*—Proceed as directed under 34.35 or 34.39, using aliquot of soln used for invert polarization, 34.117(a). If soln is clarified, only neutral Pb acetate soln may be used, and excess of Pb must be removed with dry  $\text{Na}_2\text{C}_2\text{O}_4$ .

**34.121** **COMMERCIAL GLUCOSE—OFFICIAL.—See 34.31 or 34.32****LEAD NUMBER***Canadian Lead Number (39) (Fowler Modification)—Official***34.122** **REAGENT**

*Standard basic lead acetate soln.*—Activate litharge by heating it to 650–670° for 2.5–3 hours in muffle. (Cooled product should be lemon color.) In 500 ml Erlenmeyer flask provided with return condenser boil 80 g of normal Pb acetate crystals and 40 g of the freshly activated litharge with 250 g of  $\text{H}_2\text{O}$  for 45 min. Cool, filter off any residue, and dilute with recently boiled  $\text{H}_2\text{O}$  to density of 1.25 at 20°.

## 34.123

## DETERMINATION

Weigh quantity of sirup containing 25 g of dry matter, transfer to 100 ml flask, and make up to mark at 20°, or use soln in which conductivity value has been determined, 34.129. Pipet 20 ml into large test tube, add 2 ml of the standard basic Pb acetate soln, cork, and allow to stand 2 hours.

Filter with suction on 25 ml tared Gooch, having asbestos mat at least 3 mm thick. When nearly all liquid has run thru, fill crucible with cold H<sub>2</sub>O. Repeat to total of 4 washings, taking care to prevent formation of fissures in precipitate by keeping it covered with H<sub>2</sub>O and avoiding too great suction. Dry at 100°, weigh, and multiply weight by 20.

NOTE: Filtration may be facilitated and necessity of keeping the precipitate in the crucible covered with H<sub>2</sub>O obviated by stirring weighed quantity (0.5 g or less) of dry asbestos fiber with precipitate and supernatant liquid shortly before filtration.

*Winton Lead Number (40)—Official*

## 34.124

## REAGENT

*Standard basic lead acetate soln.*—To measured volume of reagent prepared for determination of Canadian Pb number, 34.122, add 4 volumes of H<sub>2</sub>O, and filter. Run a blank with each set of determinations.

## 34.125

## DETERMINATION OF LEAD IN THE BLANK

Transfer 25 ml of the standard basic Pb acetate soln to 100 ml flask, add a few drops of acetic acid, and make up to mark with H<sub>2</sub>O. Shake, and determine PbSO<sub>4</sub> in 10 ml of the soln as directed under 34.126. Use of acetic acid is imperative in order to retain all Pb in soln when the reagent is diluted with H<sub>2</sub>O.

## 34.126

## DETERMINATION

Transfer 25 g of sample to 100 ml flask by means of H<sub>2</sub>O. Add 25 ml of the standard basic Pb acetate soln and shake. Fill to mark, shake, and allow to stand for at least 3 hours before filtering. Pipet 10 ml of the clear filtrate into 250 ml beaker, add 40 ml of H<sub>2</sub>O and 1 ml of H<sub>2</sub>SO<sub>4</sub>, shake, and add 100 ml of alcohol. Allow to stand overnight, filter on weighed Gooch crucible, wash with alcohol, dry in water oven, and ignite in muffle or over Bunsen burner, applying heat gradually at first and avoiding reducing flame. Cool, and weigh. Subtract weight of PbSO<sub>4</sub> so found from weight of PbSO<sub>4</sub> found in blank, 34.125, and multiply by factor 27.33. Use of this factor gives Pb number directly (without various calculations otherwise required).

## CONDUCTIVITY VALUE (41)—OFFICIAL

## 34.127

## APPARATUS

(a) *Conductivity cell.*—Should be made of resistance glass with platinized Pt electrodes firmly fixed and adequately protected from displacement. These electrodes may be sealed into vessel into which soln under examination may be run and subsequently drawn off (*Zerban type*), or attached to support so that they can be lowered into cylinder (or 100 ml beaker) containing the soln (dipping type). Provide the cell with thermometer graduated in tenths of degrees and covering 20–30°, and place the bulb in the immediate vicinity of electrodes. Cell constant should be ca 0.15.

(b) *Galvanometer or microphone hummer (or induction coil) and a sensitive telephone receiver.*



(c) *Suitable source of current.*—Dry or storage cells if hummer or induction coil is used; 110 volt alternating current if galvanometer is used.

(d) *Resistances of 10 and 100 ohms.*—Should be fixed and accurate.

(e) *Slide wire or Wheatstone bridge.*

(f) *Device for control of temp. of cell to within  $\pm 0.1^\circ$ .*—This may consist of thermostat or of vessel into which  $H_2O$  of suitable temp. may be run so as to adjust cell contents to  $25^\circ$ .

### 34.128

#### DETERMINATION OF THE CELL CONSTANT

Prepare solns of 0.3728 and 0.7456 g of dry KCl in  $H_2O$ , which offers a resistance of at least 25,000 ohms in the cell, and make to mark at  $20\text{--}25^\circ$  in 500 ml volumetric flasks. Fill cell with the more dilute (0.01 *M*) soln, adjust to  $25^\circ \pm 0.1^\circ$ , measure resistance, and multiply number of ohms by 141.2. Rinse with the stronger (0.02 *M*) soln, fill cell with this soln, measure its resistance at  $25^\circ$ , and multiply by 276.1. Average the 2 results.

### 34.129

#### DETERMINATION

Weigh out quantity of sirup that contains 25 g of dry matter, transfer to 100 ml volumetric flask with warm  $H_2O$  of same quality as that used in determination of cell constant, cool to  $25^\circ$ , make to mark, and measure resistance in cell at  $25^\circ \pm 0.1^\circ$ . Divide cell constant by number of ohms found.

### 34.130

#### MALIC ACID VALUE (COWLES) (42)—TENTATIVE

Weigh 6.7 g of sample into 200 ml beaker; add 5 ml of  $H_2O$ , then 2 ml of 10% Ca acetate soln; and stir. Add, gradually and with constant stirring, 100 ml of alcohol and agitate soln until precipitate settles, or let stand until supernatant liquid is clear. Filter off precipitate and wash with 75 ml of alcohol, 85% by volume. Dry filter paper and ignite in Pt dish. Add 10 ml of 0.1 *N* HCl, 43.7 and 43.8, and warm gently until all the Ca dissolves. Cool, and titrate back with 0.1 *N* NaOH soln, using methyl orange indicator. Difference in ml divided by 10 represents malic acid value of sample. Previous to use, test reagents by blank determination and apply any necessary corrections.

## SUGAR BEETS

### SUCROSE

### 34.131

#### Hot Water Digestion Method I (43).—Tentative

Pass sample (usually in form of cossettes) thru meat grinder fitted with plate having  $\frac{1}{8}$ " perforations and mix thoroly. Weigh out 26 g of prepared sample and rinse into 201.0 ml Kohlrausch flask, using ca 100 ml of  $H_2O$ . Place flask under good vacuum for 5–10 min. to remove air, carefully avoiding mechanical loss when vacuum is first applied. Add sufficient  $H_2O$  to make volume of ca 175 ml, and digest in water bath at  $80^\circ$ , supporting flask so that body is entirely immersed but is not in contact with heating element. Two or three times during digestion period remove flask, mix contents by swirling, and after each agitation wash down pulp that adheres to walls of flask with a little  $H_2O$  at  $80^\circ$ . After exactly 30 min. digestion fill flask to within 2–5 ml of mark with  $H_2O$  at  $80^\circ$  and continue digestion for exactly 10 min. longer. Cool to room temp. in water bath. Add 6 ml of basic Pb acetate soln, 34.19(a), and the small volume of  $H_2O$  at room temp. to fill to mark. (Previous additions of  $H_2O$  and reagents should be so adjusted that not over 4 ml of  $H_2O$  is required to make to volume.) Mix contents of flask well by shaking, allow to stand 5 min.,

shake again, and filter. Polarize in 400 mm glass tube, after allowing soln to stand in immediate vicinity of saccharimeter at least 5 min. before reading. If volume adjustment and polariscopic observation are made at 20°, reading gives percentage of sugar directly; if at other temps., apply formula in 34.20(a).

**NOTES:** The 1 ml over the 200 ml volume is the determined volume of marc for beets grown in Colorado and neighboring states. It should be determined for other localities.

Beets of abnormally low purity may require 8–10 ml of basic Pb acetate soln for clarification.

If trouble is experienced with foam, flask may be put under vacuum a second time after cooling, or a few drops of ether or one drop of amyl alcohol may be added before making to volume.

#### 34.132 *Hot Water Digestion Method II (44)—Tentative*

Use Ni-plated sheet Fe vessels, 11 cm high, 6 cm body diameter, and 4 cm mouth diameter, also stoppers covered with Sn foil to fit.

Weigh 26 g of prepared beet pulp, 34.131, on watch-glass (small enough to go into neck of beaker) and transfer to the metal beaker; add 177 ml of dilute basic Pb acetate soln (5 parts of basic Pb acetate soln, sp. gr. 1.25, to 100 parts of H<sub>2</sub>O); shake, and stopper lightly. Submerge beaker in water bath at 75–80° for 30 min., shaking intermittently. When all the air has been expelled (generally after 5 min.), tighten stopper. After 30 min., shake, cool to standard temp., filter, add drop of acetic acid to filtrate, and polarize in 400 mm tube. Reading is percentage of sugar in the beet pulp.

### STARCH CONVERSION PRODUCTS—TENTATIVE

#### 34.133 PREPARATION OF SAMPLE.—See 34.1

##### MOISTURE

#### 34.134 *Method I*

(Applicable to refined corn sugars)

Proceed as directed under 34.3.

#### *Method II (45)*

(Applicable to corn sirups and crude corn sugars)

#### 34.135<sup>\*</sup> APPARATUS

(a) *Filter paper.*—Strip of Whatman No. 1 filter paper, 4.375×50 cm.

(b) *Separator.*—Corrugated strip of phosphor-bronze, 1.25×40 cm. No. 36 B. & S.

(c) *Weighing bottle.*—Medium form, 40×65 mm, with 40/20 standard taper, ground-glass stopper.

#### 34.136 DETERMINATION

Place separator on filter-paper strip and roll together to form cylinder ca 30 mm in diam., fasten with paper clip, and place in weighing bottle. Dry in air oven at 100° ca 6 hours. Cool, and weigh. Remove coil and weigh ca 1 g of sirup in weighing bottle, add 1 or 2 ml of H<sub>2</sub>O, and mix, using heat if necessary. Replace coil, shaking until all soln is absorbed on paper. Dry to constant weight in vacuum oven at 100° for corn sirups or 70° for crude corn sugars (ca 6 hours).

*Method III (46)*

(Applicable to corn sirups and crude corn sugars)

## 34.137

## MATERIAL AND APPARATUS

(a) *Diatomaceous earth (Filter Cel)*.—Preferably analytical grade. If commercial grade is used, wash with HCl, then with H<sub>2</sub>O to remove acid, and dry in oven at ca 105°. (Material should give no test for acid when moistened.)

(b) *Moisture dish*.—Al moisture dish 25 mm high × 75 mm diam., with cover.

(c) *Pestle*.—Glass stirring rod ca 60 mm long with flattened end.

## 34.138

## DETERMINATION

Place 10 g of the prepared Filter Cel in moisture dish containing pestle and dry to constant weight. Weigh ca 5 g of corn sirup or sugar in nickel scoop, dilute with ca 5 ml of H<sub>2</sub>O, and run onto Filter Cel. Wash scoop with 3 successive 2 ml portions of H<sub>2</sub>O and add washings to Filter Cel. Thoroly incorporate soln with Filter Cel by means of pestle, yielding a *damp* workable mass. Dry to constant weight in vacuum oven at 100° for corn sirup or 70° for crude corn sugars.

## DRY SUBSTANCE

*Method I (47)—By Hydrometer*

## 34.139

## APPARATUS

(a) *Water bath*.—Insulated water bath with stirrer and thermostatic control, maintained at 140°F.

(b) *Cylinders*.—Pyrex, 15 × 2½", without lip.

(c) *Stopper seal*.—Consists of two rubber stoppers that fit snugly with cylinder, and are separated on a metal rod by ca 3". Rod is fixed in lower stopper but does not extend thru it. Top stopper is free to move on rod, altho sufficiently tight to maintain a predetermined position (this prevents evaporation during heating).

(d) *Baumé hydrometers*.—Streamlined type, modulus 145, standardized at 60°F. with range 35–45° Bé. in 1/10° Bé.; length overall 12–13", diam. of body 0.77–0.79"; scale length 147–155 mm.

## 34.140

## DETERMINATION

Fill cylinder with sirup to within 4" of top, taking care that sides are free from sirup. Seal cylinder with the stopper seal, placing bottom stopper within ½" of sirup surface and closing cylinder with top stopper. Immerse cylinder in water bath at 140°F so that level of sirup is ca 2" below level of H<sub>2</sub>O. Immerse hydrometer in water bath. When sirup in cylinder is free of air and has attained temp. of bath (ca 90 min.), raise cylinder until surface of sirup is at eye level. Remove stopper seal and insert hydrometer, which has been previously dried. After ca 10 min. read hydrometer. To obtain commercial Baumé, add 1° Bé. to observed reading of hydrometer:

$$\text{Com. Baumé} = \text{Bé. } 140^{\circ}/60^{\circ}\text{F} + 1^{\circ} \text{ Bé.}$$

Ascertain corresponding dry substance from 34.141.

## 34.141

*Method II (48)—By Refractometer*

(Applicable only to liquid samples containing no undissolved solids.)

Determine refractometer reading at 45°. Circulate H<sub>2</sub>O thru jackets of refractom-

eter long enough to allow temp. of prisms and of sample to reach equilibrium, continuing circulation during observation, and taking care that constant temp. is maintained. From 34.143 obtain commercial Baumé corresponding to observed refractive index. From 34.142 obtain corresponding dry substance.

**34.142 Commercial table for dry substance in corn sirup and corn sugar sirup**  
(Com. Baumé = Baumé 140°/60° F + 1.00° Bé.)

COM. BAUMÉ	DEXTROSE EQUIVALENT AND ASH							
	30.00	42.00	55.00	82.00	87.00	89.00	91.2	90.7
	0.28	0.28	0.30	0.41	0.61	0.61	0.61	1.22
	DRY SUBSTANCE (PER CENT)							
40.00	73.66	74.39	75.16	76.82	77.12	77.24	77.37	77.10
41.00	75.58	76.34	77.14	78.86	79.18	79.30	79.44	79.17
42.00	77.61	78.30	79.13	80.92	81.25	81.38	81.52	81.25
43.00	79.45	80.27	81.14	83.00	83.35	83.48	83.63	83.33
44.00	81.39	82.25	83.17	85.10	85.46	85.60	85.75	85.44
45.00	83.36	84.25	85.20	87.21	87.58	87.72	87.88	87.56
46.00	85.34	86.26	87.26	89.33	89.71	89.86	90.03	89.69
47.00	87.33	88.29	89.34	91.47	91.87	92.03	92.21	91.84

**34.143 Commercial table of refractive indices of corn sirups and corn sugar sirups at 45°**  
(Commercial Baumé = Baumé 140°/60°F.)

COM. BAUMÉ	DEXTROSE EQUIVALENT AND ASH									
	30.00	35.00	42.00	45.00	50.00	55.00	60.00	65.00	82.00	89.00
	0.28	0.28	0.28	0.28	0.30	0.30	0.30	0.30	0.41	0.61
	REFRACTIVE INDEX AT 45° C.									
40.00	1.4774	1.4773	1.4771	1.4770	1.4769	1.4768	1.4767	1.4766	1.4762	1.4760
41.00	1.4825	1.4824	1.4822	1.4821	1.4820	1.4820	1.4818	1.4817	1.4813	1.4811
42.00	1.4878	1.4877	1.4875	1.4874	1.4873	1.4873	1.4871	1.4869	1.4865	1.4863
43.00	1.4933	1.4931	1.4929	1.4928	1.4927	1.4926	1.4924	1.4923	1.4919	1.4916
44.00	1.4986	1.4985	1.4983	1.4982	1.4981	1.4980	1.4978	1.4977	1.4973	1.4971
45.00	1.5041	1.5040	1.5038	1.5037	1.5036	1.5036	1.5034	1.5033	1.5029	1.5027
46.00	1.5098	1.5097	1.5095	1.5094	1.5093	1.5092	1.5090	1.5089	1.5085	1.5083
47.00	1.5155	1.5154	1.5152	1.5151	1.5150	1.5149	1.5148	1.5147	1.5143	1.5142

**34.144** ASH.—See 34.9

(For most corn sugars and sirups it is unnecessary to re-ash)

**34.145** ACIDITY

Weigh 50 g of sample, dissolve in 200 ml of H<sub>2</sub>O, and titrate with 0.1 N NaOH, using phenolphthalein indicator to faint pink end point. (This corresponds to electro-metric pH of 8.3.) Calculate acidity as HCl.)

**34.146** HYDROGEN ION CONCENTRATION

Prepare a soln of sample containing 40% total solids and determine pH electrometrically. (Buffer capacity of product is normally such that no special provision need be made with regard to the H<sub>2</sub>O used for dilution. If glass electrode is used, standardize against reference buffer within 1.0 of determined pH.)

**34.147** NITROGEN.—See 2.24, 2.25, and 2.26

**34.148** TOTAL REDUCING SUGARS

(a) Lane-Eynon General Volumetric Method.—See 34.35.

(b) Munson and Walker General Method.—See 34.39.

## DEXTROSE (STEINHOFF METHOD)—TENTATIVE

*Zerban-Sattler modification (49)*

## 34.149

## REAGENTS

- (a) *Soxhlet modification of Fehling copper soln.*—See 34.33(a).  
 (b) *Sodium acetate soln.*—Dissolve 500 g of  $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$  in ca 800 ml of hot  $\text{H}_2\text{O}$ , cool, and make to 1 liter.  
 (c) *Potassium iodide-iodate soln.*—Dissolve 5.4 g of  $\text{KIO}_3$  and 60 g of KI in  $\text{H}_2\text{O}$ , add 0.25 g of NaOH dissolved in a little  $\text{H}_2\text{O}$ , and make to 1 liter.  
 (d) *Sulfuric acid.*—Approximately 2 N. Dilute 57 ml of  $\text{H}_2\text{SO}_4$  to 1 liter.  
 (e) *Saturated potassium oxalate soln.*—Dissolve 165 g of  $\text{K}_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$  in 500 ml of hot  $\text{H}_2\text{O}$ , and cool.  
 (f) *Sodium thiosulfate soln.*—0.1 N. Dissolve ca 25 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and make to 1 liter. Standardize against pure Cu as directed under 34.41.  
 (g) *Sugar soln.*—Dissolve a quantity of sample containing ca 10 g of solids in  $\text{H}_2\text{O}$  and dilute to 1 liter.

## 34.150

## DETERMINATION

Transfer 10 ml of the Soxhlet soln, 20 ml of the  $\text{NaC}_2\text{H}_3\text{O}_2$  soln, 10 ml of the sugar soln, and 10 ml of  $\text{H}_2\text{O}$  to 250 ml Erlenmeyer flask. Mix contents, and close flask with rubber stopper provided with Bunsen valve. Immerse in briskly boiling water bath and allow to remain for exactly 20 min. Immerse in cold running  $\text{H}_2\text{O}$ , venting valve to prevent boiling caused by vacuum. After cooling, add 25 ml of the KI- $\text{KIO}_3$  soln by pipet and mix by gentle shaking. Add rapidly 40 ml of the 2 N  $\text{H}_2\text{SO}_4$  from graduated cylinder, then add 20 ml of the  $\text{K}_2\text{C}_2\text{O}_4$  soln from graduated cylinder. Shake contents until precipitate is completely dissolved, and titrate excess I with 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$ .

Run a blank substituting  $\text{H}_2\text{O}$  for the sugar soln. Difference between titer of blank and that of sample is direct measure of  $\text{Cu}_2\text{O}$  precipitated. From 34.151 obtain dextrose equivalent corresponding to titer of 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$ .

*Correction for reducing effect of maltose.*—If maltose is present, correct observed titer of 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$  for reducing effect of maltose by subtracting correction obtained from 34.151 by interpolation.

## 34.151

*Zerban-Sattler table for determination of dextrose with copper acetate reagent\**

TITER	DEXTROSE (MG)										MALTOSE CORRECTIONS (SUBTRACT FROM OBSERVED TITER)		
											MALTOSE PRESENT (MG)		
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	200	100	50
10	25.7	26.0	26.3	26.6	26.9	27.2	27.5	27.8	28.1	28.4	2.5	1.4	0.6
11	28.7	29.0	29.3	29.6	29.9	30.3	30.6	30.9	31.2	31.5	2.3	1.2	.4
12	31.8	32.2	32.5	32.9	33.2	33.6	34.0	34.3	34.7	35.0	2.2	1.1	.4
13	35.4	35.8	36.1	36.5	36.8	37.2	37.6	37.9	38.3	38.6	2.0	1.0	.3
14	39.0	39.4	39.9	40.3	40.7	41.2	41.6	42.0	42.4	42.9	1.9	1.0	.3
15	43.3	43.8	44.2	44.7	45.1	45.6	46.1	46.5	47.0	47.4	1.8	1.0	.3
16	47.9	48.4	49.0	49.5	50.1	50.6	51.1	51.7	52.2	52.8	1.7	1.0	.3
17	53.3	53.9	54.5	55.2	55.8	56.4	57.0	57.6	58.3	58.9	1.6	0.9	.3
18	59.5	60.2	60.9	61.6	62.3	63.1	63.8	64.5	65.2	65.9	1.4	.8	.3
19	66.6	67.4	68.2	69.0	69.8	70.6	71.4	72.2	73.0	73.8	1.2	.7	.3
20	74.6	75.6	76.5	77.5	78.4	79.4	80.3	81.3	82.2	83.2	1.0	.6	.2
21	84.1	85.2	86.3	87.4	88.5	89.6	90.6	91.7	92.8	93.9	0.6	.4	.2
22	95.0										.4	.3	.1

\* The table may be interpolated for hundredths of a ml, but should not be extrapolated. *Ind. Eng. Chem., Anal. Ed.*, 10, 669 (1938); *Z. Spiritusind.*, 56, 64 (1933).

*Sichert-Bleyer modification(50)*

## 34.152

## REAGENTS

- (a) *Soxhlet modification of Fehling copper soln.*—See 34.33(a).  
 (b) *Sodium acetate soln.*—See 34.149(b).  
 (c) *Ferric ammonium sulfate soln.*—Dissolve 120 g of  $\text{Fe}_2(\text{SO}_4)_3 \cdot (\text{NH}_4)_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$  and 100 ml of  $\text{H}_2\text{SO}_4$  in  $\text{H}_2\text{O}$  and make to 1 liter.  
 (d) *Potassium permanganate soln.*—0.1 N. Dissolve ca 3.16 g of  $\text{KMnO}_4$  in  $\text{H}_2\text{O}$  and make to 1 liter. See also 34.43(a).  
 (e) *Sugar soln.*—See 34.149(g).

## 34.153

## STANDARDIZATION

To obtain factor for 0.1 N  $\text{KMnO}_4$  perform analysis as directed under 34.154 on 10 ml of soln containing 50 mg of pure dextrose. From 34.155 a titer of 15.38 ml corresponds to 50 mg of dextrose; 15.38 divided by the titer obtained gives correction factor for the  $\text{KMnO}_4$  soln. Multiply all titers by this factor before referring to 34.155. The factor should be redetermined each day analyses are made.

## 34.154

## DETERMINATION

Transfer 10 ml of the Soxhlet soln, 20 ml of the  $\text{NaC}_2\text{H}_3\text{O}_2$  soln, 10 ml of the sugar soln, and 10 ml of  $\text{H}_2\text{O}$  to 250 ml Erlenmeyer flask. Mix contents and close flask with rubber stopper provided with Bunsen valve. Immerse in boiling water bath for exactly 20 min. Filter  $\text{Cu}_2\text{O}$  precipitate thru Gooch crucible prepared as directed under 34.38, and wash flask and crucible three times with hot  $\text{H}_2\text{O}$ . (It is not necessary to remove all precipitate from flask.) Transfer asbestos mat and crucible to 150 ml beaker having mark at 60 ml. Wash flask with exactly 20 ml of the  $\text{FeNH}_4(\text{SO}_4)_2$  soln in 3 portions and transfer quantitatively to beaker containing crucible. All precipitate must be dissolved. Finally wash flask and crucible with hot  $\text{H}_2\text{O}$  and remove crucible. Add hot  $\text{H}_2\text{O}$  to 60 ml mark. Heat soln to boiling on hot plate, let stand 3 min., and titrate with the 0.1 N  $\text{KMnO}_4$ . Addition of 1 ml of  $\text{H}_3\text{PO}_4$  toward end of titration facilitates reading of end point. The pink-gray end point persists for ca 20 sec. Multiply titer by factor and obtain mg of dextrose from 34.155.

## 34.155

*Sichert-Bleyer table for determination of dextrose\**

TITER 0.1 N PERMAN- GANATE	MILLIGRAMS OF DEXTROSE (MG)									
	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
ml										
10	26.5	26.8	27.1	27.4	27.8	28.1	28.4	28.7	29.0	29.3
11	29.7	30.0	30.4	30.7	31.1	31.5	31.8	32.2	32.6	32.9
12	33.3	33.7	34.1	34.5	34.9	35.4	35.8	36.2	36.6	37.0
13	37.4	37.9	38.4	38.8	39.3	39.8	40.3	40.7	41.2	41.7
14	42.2	42.7	43.2	43.8	44.3	44.9	45.4	46.0	46.5	47.0
15	47.6	48.2	48.8	49.4	50.1	50.7	51.3	51.9	52.5	53.2
16	53.8	54.5	55.2	55.9	56.6	57.3	58.0	58.7	59.4	60.2
17	60.9	61.7	62.5	63.3	64.1	64.9	65.7	66.5	67.4	68.2
18	69.0	69.9	70.9	71.9	72.8	73.8	74.8	75.7	76.7	77.6
19	78.6	79.6	80.7	81.7	82.7	83.7	84.8	85.8	86.8	87.8
20	88.9	90.0	91.2	92.3	93.5	94.7	96.0	97.2	98.5	99.7

\* The table may be interpolated for hundredths of a ml, but should not be extrapolated. *Z. Anal. Chem.*, 107, 328 (1936); *Z. Spiritusind.*, 56, 64 (1933).

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## 35. PROCESSED VEGETABLE PRODUCTS

### CANNED PRODUCTS

#### 35.1

##### PREPARATION OF SAMPLE—OFFICIAL

(a) *Products composed of solid and liquid portions.*—Weigh full can, open, pour entire contents on round sieve with No. 8 standard screen (diam. of wire 0.84 mm and size of opening 2.38 mm) (1). Use sieve 8" in diam. for No. 3 or smaller can, and sieve 12" in diam. for cans larger than No. 3. Without shifting product, so incline sieve as to facilitate drainage of liquid.

Allow material on sieve to drain 2 min., weigh either drained solids or free liquid direct, and reweigh dry empty can. From weights thus obtained determine percentage of liquid and of solid contents. If only solid portion is required for analysis or examination, thoroly grind drained vegetables in mortar or food chopper. If composite of solid and liquid portion is required, thoroly grind entire contents of can in mortar or food chopper. In all cases, thoroly mix portion used and preserve balance in glass-stoppered containers. Unless analysis is to be completed in reasonably short time, determine moisture in portion of sample prepared as above, and to prevent decomposition dry remainder, grind, mix thoroly, and preserve in glass-stoppered containers. (Second moisture determination is required in this procedure.)

(b) *Comminuted products (tomato juice, tomato catsup, strained vegetables)* (2).—Shake unopened container thoroly to incorporate any sediment. Transfer entire contents to large glass or porcelain dish, and mix thoroly, continuing stirring for at least 1 min. Transfer well mixed sample to glass-stoppered container and shake or stir thoroly each time before removing portions for analysis.

#### 35.2

##### TOTAL SOLIDS—OFFICIAL

Weigh into flat-bottomed dish a portion of sample of such size that dry residue will not be less than 9 mg nor more than 12 mg/sq. cm. of drying surface. Distribute thinly in even layer over bottom of dish, diluting with  $H_2O$  if necessary to facilitate distribution. Place in vacuum oven at 70° with release cock left partly open so that degree of vacuum does not exceed 450 mm of Hg and moisture evolved is carried off rapidly. Dry air admitted thru release cock by bubbling thru  $H_2SO_4$ . After one hour examine dishes and remove from oven any in which material has reached apparent dryness. Continue this removal of dishes with dried material at subsequent half-hour intervals. After material in all dishes has reached apparent dryness return dishes to oven, nearly close release cock so that ca 2 bubbles of air/second are admitted thru the  $H_2SO_4$  and dry at 70° for 4 hours at pressure not exceeding 100 mm.

#### 35.3

##### INSOLUBLE SOLIDS—TENTATIVE

Wash 20 g of sample repeatedly with hot  $H_2O$ , centrifuging after each addition of  $H_2O$  and pouring clear, supernatant liquid thru weighed paper filter on Büchner funnel. (Filter used is one of two such papers dried 2 hours at 100° and weighed in covered dish. Use second paper, if necessary, when first paper becomes clogged.) After 4 or 5 washings, transfer remaining insoluble matter to filter, dry in covered dish for 2 hours at 100°, cool in desiccator, and weigh.

#### 35.4

##### SOLUBLE SOLIDS—TENTATIVE

Percentage of total solids—percentage of insoluble solids = percentage of soluble solids.



## 35.5

## SPECIFIC GRAVITY (3)—TENTATIVE

(Applicable to comminuted tomato products)

Determine sp. gr. at 20/20°, using National Canners Association sp. gr. bottle. Clean and calibrate bottle at 20° as directed under 16.3, but since bottle is not provided with cap, strike off excess H<sub>2</sub>O with straight edge, wipe bottle dry, and weigh immediately. Cool sample to 16–18°, fill flask with the pulp, and centrifuge 1 min. at ca 1000 r.p.m. Add sufficient pulp to fill flask to top and centrifuge again. Remove flask and take temp. of pulp, inserting thermometer so that no air is introduced. When temp. is just 20°, remove thermometer, add sufficient pulp at same temp. to have flask slightly overfull, and strike off even with straight edge. Clean outside of flask and weigh at once to nearest 0.01 g. Sp. gr. = weight of pulp in flask ÷ weight of H<sub>2</sub>O at 20° that flask holds.

## 35.6

ASH—OFFICIAL.—See 34.9 or 34.10

## 35.7

ALKALINITY OF ASH—OFFICIAL

Proceed as directed under 26.10. Express result as number of ml of normal acid required to neutralize ash from 100 g of sample.

## SODIUM CHLORIDE

## 35.8

*Method I—Official*

Proceed as directed under 12.42 or 12.44, using HNO<sub>3</sub> soln of ash (12.41). Calculate and report result as per cent of NaCl.

## 35.9

*Method II (Rapid Method) (4)—Tentative*

Weigh ca 5 g of material, transfer with 80% alcohol to 100 ml volumetric flask, and add sufficient 80% alcohol to give volume of ca 50 ml. Shake well to get all tomato material into suspension. Add 1 ml of HNO<sub>3</sub> and by means of pipet add an excess of 0.1 N AgNO<sub>3</sub>. Make to 100 ml with alcohol. Transfer mixture to centrifuge bottle and centrifuge 5 min. at ca 1800 r.p.m. Pipet 50 ml of supernatant liquid into 300 ml of Erlenmeyer flask, add 2 ml of saturated FeNH<sub>4</sub>(SO<sub>4</sub>)<sub>2</sub> soln and 2 ml of HNO<sub>3</sub>, and titrate to permanent light brown color with 0.1 N NH<sub>4</sub>CNS. Divide number of ml of 0.1 N AgNO<sub>3</sub> used by 2 and subtract number of ml of NH<sub>4</sub>CNS used. Multiply difference by 0.005843 to obtain weight of chlorides present expressed as g of NaCl.

## 35.10

REDUCING SUGARS BEFORE INVERSION—OFFICIAL

Weigh 20 g of sample into 200 ml flask, dilute with ca 100 ml of H<sub>2</sub>O, clarify with slight excess of neutral Pb acetate soln, 34.19(d), dilute to mark, and filter. Remove excess of Pb with anhydrous Na<sub>2</sub>SO<sub>4</sub> or with dry K oxalate. Filter, and determine reducing sugars as directed under 34.39. Express result as percentage of invert sugar.

## 35.11

REDUCING SUGARS AFTER INVERSION—OFFICIAL

Transfer 50 ml of filtrate, 35.10, to 100 ml flask, add 5 ml of HCl, and let stand overnight, as directed under 34.24(c). Nearly neutralize with NaOH soln, cool, dilute to mark, and determine reducing sugars in an aliquot as directed under 34.39. Express result as percentage of invert sugar.

## 35.12

SUCROSE—OFFICIAL.—See 34.30.

## 35.13

## TOTAL ACIDS—OFFICIAL

Proceed as directed under 26.28, using 5 g of sample. Express result as number of ml of normal alkali required to neutralize 100 g of sample.

## 35.14

## PRESERVATIVES AND ARTIFICIAL SWEETENERS.—See Chap. 32

## 35.15

## COLORING MATTERS.—See Chap. 21

## 35.16

## METALS.—See Chap. 29

## 35.17

## ALCOHOL-INSOLUBLE SOLIDS IN CANNED PEAS AND CANNED DRIED PEAS (6)—OFFICIAL

Pour sample on 8-mesh screen, using 8" screen for containers of less than 3 lbs. net weight, and 12" screen for larger quantities. Spread peas evenly and allow to drain. Transfer peas to white pan and remove any foreign material. Add volume of H<sub>2</sub>O equal to double volume of original sample.

Pour peas back on screen, spreading evenly; tilt screen as much as possible without shifting peas; and drain for 2 min. With a cloth wipe surplus moisture from lower surface of screen. Grind drained peas in food chopper until cotyledons are reduced to smooth homogeneous paste, stir, and weigh 20 g of ground material into 600 ml beaker. Add 300 ml of 80% alcohol, stir, cover beaker, and bring to boil. Simmer slowly 30 min.

Fit into Büchner funnel a filter paper of appropriate size (previously prepared by drying in flat-bottomed dish 2 hours at temp. of boiling H<sub>2</sub>O, covering with tight-fitting cover, cooling in desiccator, and weighing at once). Apply suction and transfer contents of beaker to the Büchner funnel, in such manner as to avoid running over edge of paper. Suck dry and wash material on filter with 80% alcohol until washings are clear and colorless.

Transfer filter paper and alcohol-insoluble solids to dish used in preparation of filter paper, dry uncovered for 2 hours at temp. of boiling H<sub>2</sub>O, place cover on dish, cool in desiccator, and weigh at once. From this weight deduct weight of dish, cover, and paper. Calculate this weight to percentage.

## 35.18

## FIELD CORN IN CANNED MIXTURES OF FIELD AND SWEET CORN (6)—TENTATIVE

Empty contents of No. 2 can, or representative equivalent portion of larger can, into large beaker and remove liquor and debris from fragments of kernels by flotation with cold H<sub>2</sub>O. Place upon flat plate all kernels to which outer seed coat is still attached, mix thoroly, and quarter to ca 400 pieces. Harden selected pieces in alcohol and quarter again to obtain ca 100 fragments. Cut each fragment thru with section razor or knife and avoid contamination of fragments with dextrin by washing and drying instrument after each cut. With dissecting needle remove portion ca 1/16" in diam. from uncontaminated interior of each kernel and place pieces in separate depressions of white spot plate. Cover each piece with freshly prepared I stain (0.2 g of I, 1.5 g of KI in 100 ml of H<sub>2</sub>O) and allow to stand 10 min. Brown cloud will disseminate from the portions of sweet corn due to dextrin, while soln surrounding the field corn will remain clear and the portion will be blue black and sharply outlined. Crush field corn portions to insure absence of dextrin and count those found to contain none. Use care in interpreting results, because kernels of immature sweet corn do not contain enough dextrin to produce the dense brown coloration characteristic of more mature sweet corn. In case of doubt, report as field corn only those kernels having firm texture and showing no brown coloration with

I soln on 2 confirmatory tests. Calculate percentage of field corn from total number of kernels examined.

#### LACTIC ACID—OFFICIAL

##### 35.19

##### PREPARATION OF SOLUTION

Weigh 50 g of ground and mixed sample into tared centrifuge bottle and add 100 ml of  $H_2O$ . Make acid with normal  $H_2SO_4$  against congo red paper. Adjust weight of contents of bottle to 200 g by addition of  $H_2O$ , shake vigorously, and centrifuge. Decant supernatant liquid and weigh 100 g into 100–110 ml volumetric flask. Make to 110 ml mark with  $H_2O$ , shake, and pipet 50 ml into the continuous extractor (Fig. 30). Add 0.5 ml of  $H_2SO_4$  (1+1) and 2 ml of 20%  $Na_2WO_4 \cdot 2H_2O$  soln and proceed as directed in 22.13.

If sample contains acetic acid added in course of manufacture, such as in case of catsup, transfer extracted material, after evaporation of ether, to beaker, add ca 50 ml of  $H_2O$ , and evaporate to 20 ml. Again add 50 ml of  $H_2O$  and evaporate to 20 ml. Neutralize with saturated  $Ba(OH)_2$  soln and proceed as directed under 22.13.

#### VOLATILE FATTY ACIDS IN VEGETABLE JUICES—OFFICIAL

##### 35.20

##### PREPARATION OF SOLUTION

Weigh 200 g into tared 500 ml, wide-mouth Erlenmeyer flask. Add 10 ml of normal  $H_2SO_4$  and mix. Add sufficient  $AgClO_4$  soln (1+1) to precipitate chlorides (10 ml is usually sufficient), make to 300 g with  $H_2O$ , and shake ca 1 min. Filter thru folded filter paper or centrifuge. Weigh 200 g of liquid into 200 ml volumetric flask make to mark, and shake.

##### 35.21

##### DETERMINATION

Transfer 150 ml of the prepared soln to distillation flask, Chap. 24, Fig. 37, and determine volatile acids as directed under 24.11. Mg of formic or acetic acid determined as present in distillation flask is that in 100 g of sample.

#### DRIED PRODUCTS

##### 35.22

##### MOISTURE—TENTATIVE

Mix sample within its container or with minimum exposure to air. Remove ca 50 g and immediately pass thru Wiley mill, or similar mill, equiped with 20-mesh screen. (This operation should not require more than 2 min.) Transfer ground material to tightly closed container. Spread 5–10 g of prepared sample as evenly as possible over bottom of metal dish (ca 8.5 cm in diam. and provided with tightly fitted cover), weigh, and dry 6 hours at  $70^\circ$  under pressure not exceeding 100 mm of Hg. During drying admit to oven slow current of air (ca 2 bubbles/second) dried by passing thru glass tube of 4 mm I.D. immersed 3" below surface of  $H_2SO_4$ . Replace cover, cool dish in desiccator, and weigh as soon as cool.

#### SELECTED REFERENCES

- (1) U. S. Bur. Standards Specifications for Sieves, U. S. Standard Sieve Series.
- (2) U. S. Dept. Agr. Bur. Chem. Bull. 162, p. 124.
- (3) N.C.A. Bull. 21 L, p. 36.
- (4) J. Assoc. Official Agr. Chem., 22, 765 (1939).
- (5) Ibid., 21, 90 (1938); 22, 87 (1939).
- (6) Ibid., 12, 39 (1929); 15, 167 (1932).

## 36. VITAMINS

### VITAMIN A

#### VITAMIN A IN FISH LIVER OILS (1)

##### 36.1

##### APPARATUS

(a) *Glassware*.—Use amber glassware unless tests are made to indicate the suitability of ordinary glassware under existing lighting conditions.

(b) *Spectrophotometer*.—Use any reliable spectrophotometer with any recognized ultraviolet source other than an incandescent lamp (the incandescent lamp may not be a suitable source under 320  $m\mu$ ). A direct reading photoelectric spectrophotometer equipped with continuous spectrum source reading to 220  $m\mu$  is recommended. Matched quartz cells with internal light path equal to 1 cm are preferable, but cells of other materials may be used provided they are sufficiently transparent. The cells should either be matched or suitable corrections made.

##### 36.2

##### REAGENTS

50% KOH soln, alcohol, absolute alcohol, isopropanol, U.S.P. Reference Standard for Vitamin A, phenolphthalein indicator soln, and ether in  $\frac{1}{4}$ -lb. cans (this ether may be redistilled if necessary to prevent vitamin A deterioration as indicated by  $E_{1\%}^{1\text{cm}}$  325  $m\mu$  values and shape of curve derived from reference standard). All solvents should be free from interfering extraneous absorption.

##### 36.3

##### SAPONIFICATION AND EXTRACTION

Boil 0.25–1 g of the accurately weighed sample with 30 ml of alcohol and 3 ml of the KOH soln 20 min. Maintain initial volume with reflux condenser with ground-glass joint (cork or rubber stoppers cannot be used) or by adding alcohol during boiling. Cool, add 30 ml of  $H_2O$ , and extract with 30 ml of ether in separator. Make 2 additional extractions with 2 other 30 ml quantities of ether. Combine all ether extracts in separator and pour 100 ml of  $H_2O$  without agitation thru ether layer. After allowing mixture to stand 2 min., separate and discard this  $H_2O$ . (No subsequent aqueous portions should be separated until 2 min. has been allowed for separation.) Shake vigorously with 3–5 ml of  $H_2O$ . Discard aqueous portion. If somewhat resistant emulsion forms in aqueous portion, dilute with 100 ml of  $H_2O$  to eliminate or decrease this emulsion before discarding aqueous portion. Rinse with 2 additional 3–5 ml portions of  $H_2O$  with vigorous agitation, and pour 100 ml of  $H_2O$  thru ether soln without agitation following each of the agitated rinses. Pour 100 ml of  $H_2O$  thru ether portion as final rinse. After separation of final 100 ml portion of  $H_2O$ , test for complete rinsing with phenolphthalein. Rinse further if indicator is pink. After rinses are completed, allow remaining ether extract to stand 10 min. Discard any  $H_2O$  that separates. Transfer ether extract to 100 ml beaker and evaporate on steam bath to volume of 3–5 ml (never to dryness). Transfer this residue to suitable volumetric flask, and make to volume with alcohol or isopropanol for the spectrophotometric examination.

##### 36.4

##### SPECTROPHOTOMETRIC DENSITY DETERMINATION

The potency of the oil usually is such that at least 2 dilutions are required. Aliquots from the initial soln may be taken and adjusted for final spectrophotometric readings. So adjust final volume that spectrophotometer readings lie between 10

and 40% transmittancy, corresponding to density values between 1 and 0.4, respectively. Transfer final soln to matched and corrected absorption cells for spectrophotometer readings at 5–10  $m\mu$  intervals from 270 to 450  $m\mu$ .

## 36.5

## CALCULATIONS

Calculate the extinction coefficient,  $E_{1\%}^{1cm}$  325  $m\mu$ , by dividing the optical density of the soln by length of the cell (in cm) and by concentration in per cent, where per cent is expressed in terms of original weight of oil in g/100 ml in the final soln. This value  $\times$  constant factor (2000) = the spectrophotometric value expressed in vitamin A units/g.

Use the table, 36.6, for converting transmittancy values to density values.

If the oil has not been contaminated by interfering substances, or if there has been no substantial deterioration of vitamin A, the value obtained will indicate vitamin A content of the oil. Until there is better agreement in the extinction coefficient-biological potency relationship, no precise conversion factor can be designated. Until such time, units as above indicated should be regarded as spectrophotometric vitamin A units.

## CAROTENE\* (§)

## 36.7

## REAGENTS

(a) *Alcoholic potash soln.*—Dissolve 12 g of KOH in 100 ml of alcohol.

(b) *Petroleum benzine.*—Use petroleum benzine or Skellysolve F, b.p. 30–70°.

(c) *Methanol.*—90%. Place 100 ml of  $H_2O$  in 1 liter volumetric flask and dilute to volume with absolute methanol.

(d) *Standard carotene soln.*—Purify commercial crystalline carotene as follows: Dissolve 0.1 g in 2 or 3 ml of  $CHCl_3$ , precipitate with 25 ml of methanol, filter, wash once with methanol, and dry in vacuum desiccator for not more than 1 hour. Dissolve 20 mg of the purified carotene in 1–2 ml of  $CHCl_3$  and dilute to 100 ml with petroleum benzine.

(e) *Activated magnesium carbonate.*—Place 2.5 g of U.S.P. light  $MgCO_3$  in 2 oz. bottle. Add 50 ml of the purified carotene soln, stopper tightly, shake vigorously 1 min., and centrifuge without removing stopper. Determine carotene. If no carotene is lost, the  $MgCO_3$  is suitable for use; if more than 1% carotene is lost, thoroly mix 50 g of the  $MgCO_3$  with 10 ml of  $H_2O$ . Test mixture with the carotene soln. If carotene is still removed, add 5 ml more of  $H_2O$ , and test again. Add  $H_2O$  in 5 ml portions until the  $MgCO_3$  no longer adsorbs carotene.

(f) *Calcium hydroxide.*— $Ca(OH)_2$  or commercial hydrated lime low in Mg. Sift thru 48–65-mesh sieve.

(g) *Potassium dichromate soln.*—0.02%. Weigh accurately 0.2000 g of  $K_2Cr_2O_7$ , dissolve in ca 100 ml of  $H_2O$ , and dilute to 1000 ml.

(h) *Potassium dichromate soln.*—0.10%.

## I. Phasic Separation

## 36.8

## PREPARATION OF SOLUTION

(a) *Hay and dried plants.*—Weigh out 1–5 g of sample, transfer to 200 ml Erlenmeyer flask, and for each gram of sample add 20 ml of the freshly prepared KOH soln. Fit flask with reflux condenser and boil contents on steam bath or hot plate 30 min. If portions of sample collect on sides of flask, wash down with alcohol from

\* Phasic separation method for carotene in hay and dried plants official, first action; all other method tentative. See also 12.75–12.77 and 20.55–20.59.

## 36.6

Per cent transmittancy-absorbency or density values\*

<i>T</i>	<i>D</i>	<i>T</i>	<i>D</i>	<i>T</i>	<i>D</i>	<i>T</i>	<i>D</i>
0.1—3		22.5—0.648		48.5—0.314		74.5—0.128	
0.2—2.7		23.0—0.638		49.0—0.310		75.0—0.125	
0.3—2.53		23.5—0.629		49.5—0.305		75.5—0.122	
0.4—2.40		24.0—0.620		50.0—0.301		76.0—0.119	
0.5—2.30		24.5—0.611		50.5—0.297		76.5—0.116	
0.6—2.22		25.0—0.602		51.0—0.292		77.0—0.114	
0.7—2.15		25.5—0.593		51.5—0.288		77.5—0.111	
0.8—2.10		26.0—0.585		52.0—0.284		78.0—0.108	
0.9—2.05		26.5—0.576		52.5—0.280		78.5—0.105	
1.0—2.0		27.0—0.569		53.0—0.276		79.0—0.102	
1.5—1.82		27.5—0.562		53.5—0.272		79.5—0.100	
2.0—1.70		28.0—0.553		54.0—0.268		80.0—0.097	
2.5—1.60		28.5—0.545		54.5—0.264		80.5—0.094	
3.0—1.52		29.0—0.538		55.0—0.260		81.0—0.092	
3.5—1.46		29.5—0.531		55.5—0.258		81.5—0.089	
4.0—1.40		30.0—0.523		56.0—0.252		82.0—0.086	
4.5—1.35		30.5—0.516		56.5—0.248		82.5—0.084	
5.0—1.30		31.0—0.509		57.0—0.244		83.0—0.081	
5.5—1.26		31.5—0.502		57.5—0.240		83.5—0.078	
6.0—1.22		32.0—0.495		58.0—0.237		84.0—0.076	
6.5—1.19		32.5—0.486		58.5—0.233		84.5—0.073	
7.0—1.15		33.0—0.481		59.0—0.229		85.0—0.071	
7.5—1.12		33.5—0.475		59.5—0.225		85.5—0.068	
8.0—1.10		34.0—0.469		60.0—0.222		86.0—0.066	
8.5—1.07		34.5—0.462		60.5—0.218		86.5—0.063	
9.0—1.05		35.0—0.456		61.0—0.215		87.0—0.060	
9.5—1.02		35.5—0.450		61.5—0.211		87.5—0.058	
10.0—1.00		36.0—0.444		62.0—0.208		88.0—0.056	
10.5—0.979		36.5—0.438		62.5—0.204		88.5—0.053	
11.0—0.958		37.0—0.432		63.0—0.234		89.0—0.051	
11.5—0.939		37.5—0.426		63.5—0.197		89.5—0.048	
12.0—0.921		38.0—0.420		64.0—0.194		90.0—0.046	
12.5—0.903		38.5—0.415		64.5—0.190		90.5—0.043	
13.0—0.886		39.0—0.409		65.0—0.187		91.0—0.041	
13.5—0.870		39.5—0.403		65.5—0.184		91.5—0.039	
14.0—0.854		40.0—0.398		66.0—0.180		92.0—0.036	
14.5—0.839		40.5—0.393		66.5—0.177		92.5—0.034	
15.0—0.824		41.0—0.387		67.0—0.174		93.0—0.032	
15.5—0.810		41.5—0.382		67.5—0.171		93.5—0.029	
16.0—0.796		42.0—0.377		68.0—0.167		94.0—0.027	
16.5—0.783		42.5—0.372		68.5—0.164		94.5—0.025	
17.0—0.770		43.0—0.367		69.0—0.161		95.0—0.022	
17.5—0.757		43.5—0.362		69.5—0.158		95.5—0.020	
18.0—0.745		44.0—0.357		70.0—0.155		96.0—0.018	
18.5—0.733		44.5—0.352		70.5—0.152		96.5—0.015	
19.0—0.721		45.0—0.347		71.0—0.149		97.0—0.013	
19.5—0.710		45.5—0.342		71.5—0.147		97.5—0.011	
20.0—0.699		46.0—0.337		72.0—0.143		98.0—0.009	
20.5—0.688		46.5—0.333		72.5—0.140		98.5—0.007	
21.0—0.678		47.0—0.328		73.0—0.137		99.0—0.004	
21.5—0.668		47.5—0.323		73.5—0.134		99.5—0.002	
22.0—0.658		48.0—0.319		74.0—0.131		100.0—0.000	

\* Absorbency =  $2 - \log_{10} T$ , where  $T = \frac{\text{Transmission Soln}}{\text{Transmission Solvent}} \times 100$ .

wash bottle. Cool contents of flask. (Volume of petroleum benzine to be used later for extraction may be reduced by filtering directly, after cooling, thru sintered-glass funnel of No. 3 porosity, extracting residue with small portions of petroleum benzine until solvent is colorless, and proceeding as directed below.) Add 100 ml of petroleum benzine and after shaking for 1–2 min. and allowing sediment to settle, decant mixture into 500 ml separator. Repeat procedure twice more with 25 ml portions of the solvent, breaking up residue, which sometimes forms an adherent mass, by shaking with 10–15 ml of alcohol. After two or three additional extractions with 20 ml portions of solvent (soln usually comes off colorless), discard residue.

Remove xanthophyll with 25 ml portions of 90% methanol, shaking 2 min. each time. Continue these extractions until methanol is colorless (6–12 washings, depending on amount of xanthophyll in sample). Wash the petroleum benzine containing the carotene twice with 50 ml of  $H_2O$  to remove the methanol, concentrate under reduced pressure if necessary, filter into volumetric flask (of such size as to obtain convenient concentration for measurement of carotene) thru filter paper upon which is placed small amount of anhydrous  $Na_2SO_4$ , and adjust to volume. Proceed as directed in 36.11, 36.13, or 36.15.

This carotene soln may be further purified by shaking 50 ml aliquot with 2.5 g of the activated  $MgCO_3$ , 36.7(e), in 2 oz. tightly stoppered wide-mouth bottle for 1 min., and centrifuging.

(b) *Fresh green materials.*—For samples to be analyzed soon after gathering (1–2 hours), place 100 g of material in large evaporating dish, soak in 100 ml of alcohol 5 min., and cut up with scissors. Add 100 g of clean white sand (free of organic matter), and grind until uniform mixture is obtained. For samples necessitating shipment or delay in analysis, place 100 g of material in tared fruit jar with weighed quantity of alcohol or methanol. Seal jar with rubber under lid. Upon arrival at laboratory, weigh jar and contents, and to obtain weight of sample subtract weight of jar and alcohol from total weight. Pour contents of jar into evaporating dish, cut up with scissors, and grind with sand as directed above.

Decant liquid from solid part thru cheese cloth and make to volume. Weigh solids. Take aliquots of both solid and liquid, equivalent to 5 g of fresh untreated material, and mix together for carotene analysis. Saponify by boiling 30 min. in 50 ml of the KOH soln. Cool, add 50 ml of petroleum benzine, and decant liquid into separator. Transfer residue to mortar and grind with pestle, first with 15 ml portions of petroleum benzine, and then with mixture of 5 ml of alcohol and 15 ml of petroleum benzine, until no further color is extracted. Decant washings into the separator and proceed as directed in (a), beginning "Remove xanthophyll."

If crude carotene on dry basis only is desired, analyze preserved samples by pouring off as much of alcohol as possible, grinding solid portion in food chopper, weighing out 5 g and proceeding as directed in (a), determining dry matter on separate portion.

(c) *Fresh sweet potatoes.*—Proceed as directed in (a), except to substitute alcohol for the alcoholic KOH soln.

(d) *Fresh carrots, apricots, and similar materials.*—To 5 g of finely ground sample add 40–50 ml of alcohol and reflux 15 min. Filter thru fluted filter or fritted-glass funnel and wash residue with small portions of alcohol. Remove residue, add 25 ml of alcohol, and again reflux 15 min. Filter and wash as above. If residue contains more than very slight amount of yellow color, reflux a third time. To combined alcoholic solns add ca 25 ml of the KOH soln, and reflux ca 5 min. Add 50–100 ml of petroleum benzine. Shake, and then add 100–150 ml of  $H_2O$  and allow layers to separate. Draw off aqueous alcoholic layer and re-extract with 25–50 ml of petro-

leum benzene. Repeat this extraction until petroleum benzene layer separates colorless, or only slightly colored. Proceed as directed in (a), beginning "Remove xanthophyll."

(e) *Dehydrated sweet potatoes, carrots, apricots, and similar materials.*—To 1 g of finely ground sample add ca 4 ml of  $H_2O$  and allow mixture to stand 30–40 min. Add 40–50 ml of alcohol and proceed as directed in (d).

(f) *Dehydrated green leafy vegetables.*—Proceed as directed in (a).

(g) *Canned foods.*—Pour contents of can onto piece of cheesecloth and allow liquid to drain. Grind solids in food chopper and proceed as directed in (a).

(h) *Butter and other fats, egg yolks, and liver.*—Weigh 5 g of sample and proceed as directed in (a).

(i) *Blood plasma.*—Weigh 10–30 g, reflux 15 min. with 25–50 ml of the KOH soln, and proceed as directed in (a).

(j) *High lycopene-containing materials (tomatoes and watermelons).*—Place 1–2 g of the activated  $MgCO_3$ , 36.7(e), in glass tube 5–8 mm wide and ca 15 mm tall, constricted at one end and plugged with wad of cotton. Apply suction and pack the  $MgCO_3$  firmly but not too tightly with cork having smooth surface, attached to glass rod.

Weigh 5 g of sample and proceed as directed in (a). Dilute the final petroleum benzene soln of carotene to 200 ml, evaporate 50 ml aliquot in vacuo to 10 ml, and run thru the  $MgCO_3$  column. Wash column with petroleum benzene until all carotene is washed thru. (Red band of lycopene will pass slowly down column; be careful not to wash it thru the column.) Dilute soln that passes thru column to appropriate volume and proceed as directed in 36.11, 36.13, or 36.15.

## II. Chromatographic Separation

### 36.9

#### EXTRACTION

In the extraction of pigments, avoid heating because it produces neo beta carotene. For some products the phasic separation with methanol can be omitted.

(a) *Fresh green materials, butter, egg yolk, and fresh or dried fecal material.*—Chop fresh material into pieces ca  $\frac{1}{4}$ " long and weigh 10 g into chamber of Waring Blendor or similar apparatus. Add 150–200 ml of the KOH soln and grind 5 min. Proceed as directed in 36.8(a), beginning "Add 100 ml of petroleum benzene." Instead of determining carotene directly, treat the final petroleum benzene soln as directed in 36.10.

(b) *Dried hay, grass, and dehydrated leafy vegetables.*—To 5–25 g of sample, depending on carotene content, add 150–200 ml of the KOH soln and let stand overnight in refrigerator. Then grind in Waring Blendor or similar apparatus and proceed as directed in (a).

(c) *Fresh sweet potatoes.*—Proceed as directed in (a), except to use alcohol instead of the KOH soln.

(d) *Fresh carrots, apricots, and similar materials.*—Extract with alcohol as directed in (c). To the alcoholic extract add 25 ml of the KOH soln, and let stand 5 min., then extract with petroleum benzene, etc., as directed in (a).

(e) *Dehydrated fruits and vegetables high in sugar, such as dried apricots, carrots, or sweet potatoes.*—Weigh 5–10 g of sample, add 50–100 ml of  $H_2O$ , and allow to stand overnight in refrigerator. Then proceed as directed in (a).

### 36.10

#### ADSORPTION

In constricted end of glass tube 5–8 mm wide and 15–20 cm high, place small wad of cotton. Apply suction and by means of smooth-surfaced cork attached to glass rod



pack the  $\text{Ca}(\text{OH})_2$ , 36.7(f), 0.2 g at a time, into the tube gently but firmly until column ca 10 cm long is obtained.

Place 2–5 ml of petroleum benzine in column and apply suction. Before all the petroleum benzine has been drawn into column add the petroleum benzine soln of carotene from 36.9. When this has been nearly drawn into column add petroleum benzine. (Always keep surface of column covered with petroleum benzine.) Wash column with petroleum benzine until zones of pigments have separated (2–4 hours). Scrape each zone from column into beakers, using wire flattened at one end. Keep powdered material in beakers covered with petroleum benzine. Extract each pigment from powdered material by stirring with petroleum benzine containing 2% of alcohol. If  $\text{Ca}(\text{OH})_2$  does not settle readily, add alcohol dropwise until it coagulates. Decant liquid into separator and extract the  $\text{Ca}(\text{OH})_2$  again. Repeat extraction until all pigment is removed. Combine extracts and wash 3 times with  $\text{H}_2\text{O}$ , to remove alcohol. If extracts are still cloudy, wash once with  $\text{HCl}$  (1:100) and then once with  $\text{H}_2\text{O}$ . Dry over anhydrous  $\text{Na}_2\text{SO}_4$ , dilute to suitable volume in graduated flasks, and determine amount of each pigment (expressed as carotene) by one or more of methods given in 36.11, 36.13, or 36.15.

With most materials, zones of pigments found in column starting from top are as follows:

(1) *Impurity A*.—May consist of several zones of yellow, red, or brownish yellow pigments.

(2) *Neo-beta-carotene U*.—Light orange pigment just above beta-carotene zone. This pigment separates late in analysis.

(3) *Beta carotene*.—Wide reddish orange zone.

(4) *Neo-beta-carotene B*.—Yellow-orange zone immediately below beta-carotene zone.

(5) *Alpha-carotene*.—Orange zone that is not present in most materials in appreciable amounts.

Another small zone is sometimes present below the alpha carotene zone. For all practical purposes it may be removed with the alpha carotene. If zones do not separate, wash the column with petroleum benzine containing 1–5% of acetone.

#### DETERMINATION

##### 36.11

##### *Spectrophotometric method*

For each determination make optical density measurements at wave lengths of 4500, 4700, and 4800 Å. U. Using the following specific absorption coefficients calculated for beta carotene at these wave lengths, determine carotene concentration for each wave length, take average, and report results to 0.1 p.p.m.

##### 36.12

##### *Specific absorption coefficients* (Absorption of 1 g/liter in 1 cm cell)

WAVE LENGTH, Å	SKULLY SOLVNE B.P. 60–70°	PETROLEUM BENZINE B.P. 40–60°
4500	238	243
4550		231
4700	200	207
4800	212	212

## 36.13

*Colorimetric method*

Estimate amount of carotene in sample by comparing it colorimetrically against 0.1%  $K_2Cr_2O_7$  soln. Put the soln of the sample in left-hand cup of colorimeter and set scale at 0.5 cm, 1 cm, 2 cm, 3 cm, or 4 cm, according to amount of color present. Vary depth of dichromate soln in right-hand cup until density of color in both cups is equal, make eight independent readings, and record in mm. Average readings. Make the dichromate readings between 4 mm and 12 mm on colorimeter. If necessary, make a reading below 4 mm, but repeat analysis with larger sample.

By use of table, 36.14, transform the depth in mm of 0.1%  $K_2Cr_2O_7$  soln into p.p.m. of carotene. Then calculate the p.p.m. of carotene actually in sample (p), using following formula:

$$p = \frac{\text{p.p.m. of carotene (from table)} \times \text{ml of soln}}{\text{g of sample} \times \text{cm depth of sample soln}}$$

Report carotene to 0.1 p.p.m.

## 36.14

*Table for calculating carotene from 0.1%  $K_2Cr_2O_7$  readings*

0.1% $K_2Cr_2O_7$	CAROTENE	0.1% $K_2Cr_2O_7$	CAROTENE
mm	p.p.m.	mm	p.p.m.
1.0	0.5	6.6	4.1
1.2	0.7	6.8	4.2
1.4	0.8	7.0	4.3
1.6	0.9	7.2	4.5
1.8	1.0	7.4	4.6
2.0	1.2	7.6	4.7
2.2	1.4	7.8	4.8
2.4	1.5	8.0	4.9
2.6	1.6	8.2	5.0
2.8	1.7	8.4	5.2
3.0	1.8	8.6	5.3
3.2	2.0	8.8	5.4
3.4	2.1	9.0	5.6
3.6	2.2	9.2	5.8
3.8	2.3	9.4	5.9
4.0	2.5	9.6	6.0
4.2	2.6	9.8	6.1
4.4	2.7	10.0	6.3
4.6	2.8	10.2	6.5
4.8	2.9	10.4	6.7
5.0	3.1	10.6	6.8
5.2	3.2	10.8	6.9
5.4	3.4	11.0	7.1
5.6	3.5	11.2	7.3
5.8	3.6	11.4	7.4
6.0	3.8	11.6	7.5
6.2	3.9	11.8	7.6
6.4	4.0	12.0	7.8

## 36.15

*Photoelectric Colorimetric Method*

Dilute 5 ml of the standard carotene soln, 36.7(d), to 500 ml with petroleum benzene (Soln B). Then dilute 40 ml of Soln B to 50 ml (Soln C), 30 ml of Soln B to 50 ml (Soln D), 20 ml of Soln B to 50 ml (Soln E), 10 ml of Soln B to 50 ml (Soln F), and 5 ml of Soln B to 50 ml (Soln G). Read Solns B, C, D, E, F, and G, which contain 2.0, 1.6, 1.2, 0.8, 0.4, and 0.2 p.p.m. of carotene, respectively, in photoelectric

colorimeter equipped with the proper filters. Plot colorimeter readings against concentration of carotene on graph paper. Use this curve for estimating carotene in solns. If the photoelectric colorimeter does not have the sensitivity required to give proper response to above concentrations of carotene, use 6 other concentrations of carotene with ca same degree of difference as ones given.

Check colorimeter from time to time against a known concentration of the pure carotene or of the  $K_2Cr_2O_7$  soln, 36.7(g). If the latter is used, always use same light filters in the colorimeter, as carotene equivalent of  $K_2Cr_2O_7$  is not the same for all light filters. If colorimeter is more than 5% off, recalibrate.

Place the carotene solns, 36.8 or 36.10, in the colorimeter and read the color. From the colorimeter reading, locate on the calibration curve the corresponding concentration of carotene and calculate carotene in solution to 0.1 p.p.m.

### VITAMIN B

#### THIAMINE HYDROCHLORIDE (VITAMIN B<sub>1</sub>) (3)—TENTATIVE

##### *Growth Method*

#### 36.16

##### PRELIMINARY PERIOD

Thruout preliminary period each rat must be raised under immediate supervision of, or according to directions specified by, the assayer, and maintained on dietary regimen that provides for normal development in all respects, except that such dietary regimen shall subsequently allow in rats weighing 40–50 g and not exceeding 28 days of age and subsisting on suitable vitamin B<sub>1</sub>-deficient diet and H<sub>2</sub>O for interval not exceeding 50 days, the development of characteristic symptoms of vitamin B<sub>1</sub> deficiency (polyneuritis).

#### 36.17

##### DEPLETION PERIOD

A rat is suitable for depletion period when its age does not exceed 28 days, if its body weight exceeds 39 g and does not exceed 50 g, and if the animal manifests no evidence of injury, or disease, or anatomical abnormality that might hinder growth or development. Thruout depletion period provide each rat with the vitamin B<sub>1</sub> test diet and H<sub>2</sub>O *ad libitum*, and have no other dietary supplement available to the animal. Thruout depletion period and until the assay has been completed keep rats in cages provided with wire cloth bottom, each mesh of which shall be not less than 8×8 mm.

#### 36.18

##### ASSEMBLING RATS INTO GROUPS FOR ASSAY PERIOD

Assemble rats that are suitable for the assay period into groups of at least 8 animals. For each assay material have one or more assay groups. In the assay of each material, provide at least one control group and at least one reference group, but one control group and one reference group may be used for the concurrent assay of more than one assay material. For assembling rats into groups allow not more than 21 days. On any one day during interval of assembling rats into groups do not allow total number of rats that has been assigned to make up any one group to exceed by more than two the number of rats that has been assigned to make up any other group. (When assembling of all groups has been completed the total number of rats in each group, and the number of rats of one sex in each group, must be the same.) Assign not more than 3 rats from one litter to one group. When assembling of all groups has been completed, average weight of rats in any one group on day

beginning assay period must not exceed by more than 10 g the average weight of rats in any other group.

## 36.19

## ASSAY PERIOD

A rat is suitable for the assay period provided depletion period has exceeded 10 days but not 30 days, and provided the rat manifests evidence of vitamin B<sub>1</sub> deficiency characterized by stationary or declining weight. Thruout assay period keep each rat of the control, reference, and assay groups in an individual cage and provide with vitamin B<sub>1</sub> test diet and H<sub>2</sub>O *ad libitum*. Thruout the assay, administer reference material to each rat in the reference group and administer the assay material to each rat in the assay group. In any one assay administer the reference and assay material in same manner. During the assay period maintain all conditions of environment as uniform as possible with respect to the assay, reference, and control groups.

## 36.20

## RECORDING OF DATA

On day beginning depletion period, record body weight of each rat. From 7th day of depletion period until beginning of assay period, record body weight of each rat at intervals not exceeding 3 days. During assay period, record body weight of each rat at intervals not exceeding 7 days.

## 36.21

VITAMIN B<sub>1</sub> POTENCY OF THE ASSAY MATERIAL

In determining vitamin B<sub>1</sub> potency of assay material calculate performance of rats of assay and reference groups on basis of difference between average weight of each group at end of the assay period and average weight of same rats on day beginning assay period. Consider data from reference group valid for establishing the vitamin B<sub>1</sub> potency of assay material only when two-thirds or more of total number of animals comprising a reference group have made individually, between beginning day of the assay period and 28th day thereafter, an increase in body weight equaling or exceeding 12 g and not exceeding 100 g. Data for an assay group are not valid for establishing vitamin B<sub>1</sub> potency of an assay material if average weight of control group is greater at end of assay period than at beginning of assay period.

Total amount of assay material administered during assay period contains a quantity of vitamin B<sub>1</sub> equal to or greater than total amount of vitamin B<sub>1</sub> administered to reference group during assay period if that quantity promotes in the assay group an average gain in weight equal to or greater than average gain in weight produced in reference group by administration of reference material.

If reference material and assay material are administered by inclusion in the diet (option 4 below) then make comparison of quantities of reference and assay material on basis of quantity of each contained in 100 g of the supplement diets.

## 36.22

## DEFINITIONS

As herein used, unless context otherwise indicates, the term *administer* means to supply the reference material or assay material to each rat in any one of the following manners:

(1) By injecting parenterally; (2) by placing in mouth of animal daily; (3) by placing before animal daily in dish separate from vitamin B<sub>1</sub> test diet; and (4) by replacing an equal weight of sucrose in the vitamin B<sub>1</sub> test diet and intimately mixing the material with the diet.

The term *assayer* means the individual immediately responsible for interpretation of the assay; the term *assay group* means a group of rats to which assay material is administered during assay period; the term *assay material* means the material under examination for its vitamin B<sub>1</sub> content; the term *assay period* means the interval in life of a rat between last day of depletion period and 29th day thereafter; the term *assemble* means the procedure by which rats are selected and assigned to groups for purpose of feeding, care and observation; the term *control group* means a group of rats receiving no assay or reference material during the assay period; the term *daily* means 6 days of each week of the assay period; the term *depletion period* means the interval in life of a rat between last day of preliminary period and first day of assay period; the term *reference group* means a group of rats receiving reference material during assay period; the term *reference material* means U.S.P. Vitamin B<sub>1</sub> Reference Standard; the term *stationary or declining weight* means the condition of a rat when body weight on any given day is equal to or less than body weight on 5th day prior to the given day; the term *vitamin B<sub>1</sub> test diet* means a uniform mixture that has not been compounded for more than 14 days, of following food materials in proportions designated:

## 36.23

VITAMIN B<sub>1</sub> TEST DIET

	<i>per cent</i>
Sucrose	60
Casein (1)	18
Salt Mixture (2)	4
Autoclaved Yeast (3)	5
Autoclaved Peanuts (4)	10
Treated Liver Extract (5)	1
Cod Liver Oil (U.S.P.)	2
Pyridoxine	.0002

(1) Free from demonstrable traces of vitamin B<sub>1</sub>.

(2) Either salt mixture No. 1, U.S.P. XII, p. 637, or salt mixture having essentially same proportions of the elements.

(3) Autoclave in steam at 15 lbs. pressure for 5 hours layers of dried yeast not more than 6 mm in depth and then dry at a temp. not exceeding 65°.

(4) Crush unroasted, shelled, No. 1 Virginia peanuts in food chopper; autoclave in steam at 15 lbs. pressure in layers not more than 12 mm in depth; then dry at a temp. not exceeding 65°. Incorporate in the basal diet by grinding with requisite quantity of sucrose.

(5) Dissolve 100 g of liver extract in 1 liter of 0.6% NaHSO<sub>3</sub> soln. (Salt used should be tested to insure bisulfite content.) Allow soln to stand 24 hours in well-stoppered bottle; then acidify with HCl to pH 1.5, distil under reduced pressure at temp. not exceeding 50° until volume has been reduced one-half, and finally dry on vitamin B<sub>1</sub>-free casein at temp. not exceeding 65°.

*Fluorometric Method*

## 36.24

## REAGENTS AND APPARATUS

(a) *Double-normal sodium acetate*.—Dissolve 275 g of NaC<sub>2</sub>H<sub>3</sub>O<sub>2</sub>·3H<sub>2</sub>O in sufficient H<sub>2</sub>O to make 1000 ml.

(b) *Bromocresol green pH indicator*.—Dissolve 0.1 g of the indicator by triturating in agate mortar with 2.8 ml of 0.05 N NaOH, then dilute to volume of 200 ml with H<sub>2</sub>O.

(c) *Thymol blue pH indicator*.—Dissolve 0.1 g of the indicator by triturating in agate mortar with 4.3 ml of 0.05 *N* NaOH, then dilute to volume of 200 ml with  $H_2O$ .

(d) *Enzyme soln*.—Prepare, on day on which it is to be used, a 10% soln ( $H_2O$ ) of an enzyme preparation potent in diastatic and phosphorolytic activity.

(e) *Base-exchange silicate*.—Purify an artificially prepared silicate of base-exchange type, in form of granular powder of 50–80-mesh size, as follows: Place convenient quantity (100–500 g) of the base-exchange silicate in suitable beaker, add sufficient hot 3% acetic acid to cover material, and boil 10–15 min., stirring continuously. Allow mixture to settle, and decant supernatant liquid. Repeat this washing 3 times, then wash in similar manner 3 times with hot KCl soln (1 part by weight of KCl in 4 volumes of soln), and finally wash with boiling  $H_2O$  (distilled  $H_2O$  must be used) until last washing gives no reaction for Cl. Dry material at ca 100° and store in well-closed container.

(f) *Base-exchange tube*.—Use base-exchange glass tube having over-all length of 200 mm. Reservoir at upper end, 50 mm in length and 25 mm in diam., converges into the adsorption tube, which is 5–6 mm internal diam. and ca 140 mm long. At lower end, tube is drawn into capillary ca 10 mm long and of such diam. that when tube is charged the rate of flow will be not more than 1 ml/min. Prepare tube for use as follows: Place over upper end of capillary, with aid of glass rod, a pledget of fine glass wool. Add to the adsorption tube a water suspension of 1.0–2.0 g of the purified base-exchange silicate, taking care to wash down all silicate from walls of reservoir. To keep air out of adsorption column, keep layer of liquid above surface of the silicate during adsorption process. The tube may be prevented from draining by placing rubber cap (filled with  $H_2O$  to avoid inclusion of air) over lower end of capillary.

(g) *Neutral potassium chloride soln*.—Dissolve 250 g of reagent KCl in sufficient  $H_2O$  to make 1000 ml.

(h) *Acid potassium chloride soln*.—Add 8.5 ml of HCl to 1000 ml of the neutral KCl soln.

(i) *Sodium hydroxide soln*.—15%. Dissolve 15 g of NaOH in sufficient  $H_2O$  to make 100 ml.

(j) *Potassium ferricyanide soln*.—1%. Dissolve 1 g of  $K_3Fe(CN)_6$  in sufficient  $H_2O$  to make 100 ml. Prepare this soln on day it is used.

(k) *Oxidizing reagent*.—Mix 4.0 ml of the 1%  $K_3Fe(CN)_6$  soln with sufficient of the 15% NaOH soln to make 100 ml. Use this soln within 4 hours.

(l) *Isobutyl alcohol*.—Use isobutyl alcohol, reagent grade, and redistil in all glass equipment.

(m) *Quinine sulfate stock soln*.—Use quinine sulfate soln to govern reproducibility of fluorophotometer. Prepare stock soln of this reagent by dissolving 10 mg of quinine sulfate in sufficient 0.1 *N*  $H_2SO_4$  to make 1000 ml. Preserve this soln in light-resistant containers.

(n) *Quinine sulfate standard soln*.—Dilute 1 volume of the quinine sulfate stock soln with 39 volumes of 0.1 *N*  $H_2SO_4$ . (This soln fluoresces to ca same degree as does the thiochrome obtained from 1 microgram of thiamine hydrochloride.) Preserve this soln in light-resistant containers.

(o) *Thiamine hydrochloride stock soln*.—Weigh accurately 20–25 mg of U.S.P. Thiamine Hydrochloride Reference Standard that has been kept in desiccator over  $P_2O_5$  for at least 16 hours. Since reference standard is hygroscopic, take precautions to avoid absorption of moisture. Dissolve in 20% alcohol adjusted to pH of 3.5–4.3 with HCl and make to volume of 1000 ml. Store in cool place in well-closed, light-resistant container.

(p) *Thiamine hydrochloride standard soln.*—From portion of the stock soln that has been warmed to room temp., transfer to 100 ml volumetric flask an aliquot containing exactly 100 micrograms of thiamine hydrochloride, and dilute to 100 ml with  $H_2O$  adjusted to pH of 3.5–4.3 with HCl. (Each ml of this soln contains 1 microgram of thiamine hydrochloride.) Treat dilutions of this soln as directed in 36.25 with respect to acid digestion, enzyme treatment, adsorption, elution from the base exchange silicate, oxidation, and measurement of fluorescence.

### 36.25

#### PREPARATION OF ASSAY SOLUTION

Take such quantity of material for the assay that ratio of volume of 0.1  $N H_2SO_4$  used for extraction to quantity of sample is at least 15 to 1, and that content of thiamine is equivalent to 30–100 micrograms of thiamine hydrochloride. (For samples of cereal and vegetable materials the proper quantity of sample is that which has thiamine content equivalent to 5–30 micrograms.) Place the accurately weighed material in 65 ml of 0.1  $N H_2SO_4$  contained in 100 ml centrifuge tube and digest on steam bath, with frequent mixing, 30 min. If liquid is not distinctly acid to the thymol blue pH indicator, add sufficient 20%  $H_2SO_4$  to make it acid. Cool, and adjust pH to 4–4.5 by the addition of the 2  $N NaC_2H_3O_2$  soln, using bromocresol green pH indicator on spot plate. Add 5 ml of the enzyme soln, mix, and incubate at 45–50° for 3 hours. Cool, centrifuge mixture until supernatant liquid is clear or practically so, and quantitatively transfer supernatant liquid to 100 ml volumetric flask. Wash residue by centrifuging with 10 ml, then with 5 ml of 0.1  $N H_2SO_4$ . Add washings to supernatant liquid and dilute to 100 ml with  $H_2O$ .

Pass thru the prepared base-exchange tube an aliquot of the soln estimated to contain 5–10 micrograms of thiamine, and wash tube with three 5 ml portions of almost boiling  $H_2O$ , taking care to prevent surface of liquid from falling below surface of silicate.

Elute the thiamine from the base-exchange silicate by passing successively thru the tube three 5 ml portions of almost boiling acid KCl soln. Collect 15 ml of the liquid (eluate) in glass-stoppered, 25 ml volumetric flask, cool, and dilute to volume of 25 ml with acid KCl soln. This constitutes the assay soln.

### 36.26

#### OXIDATION OF THIAMINE TO THIOCHROME AND MEASUREMENT OF FLUORESCENCE

Determine thiamine content of the oxidized assay soln by comparing the intensity of fluorescence of an extract of this soln exposed to ultraviolet rays ranging from 350 to 400  $m\mu$  with that from the oxidized thiamine hydrochloride standard soln. Intensity of the fluorescence is proportional to quantity of thiamine present and may be measured with aid of various instruments.

Add to quantities of the assay soln and of the similarly treated thiamine hydrochloride standard soln containing 0.10–2.0 micrograms of thiamine, sufficient acid KCl soln to produce volume of 5 ml; then add without undue mixing (in 2 seconds) 3 ml of oxidizing reagent. Immediately thereafter add 13 ml of isobutyl alcohol and shake vigorously at least 1½ min. Centrifuge mixture at low speed until clear supernatant soln is obtained. Measure in fluorometer the intensity of fluorescence of the isobutyl alcohol soln directly if clear, or if cloudy, after shaking with 2 g of anhydrous  $Na_2SO_4$ . Compare with fluorescence produced after oxidation of the properly prepared thiamine hydrochloride standard soln. Use quinine sulfate standard soln to govern reproducibility of instrument, and make correction for fluorescence produced by substances other than thiamine by determining intensity of fluorescence of

thiamine hydrochloride standard soln, and assay solns treated as described above, but with 15% NaOH soln replacing the oxidizing reagent.

The thiochrome assay for thiamine hydrochloride is applicable to a number of materials, but cannot be relied upon when certain interfering substances are present. In the latter case, use the biological assay, 36.16–36.23.

### Fermentation Method

#### 36.27

#### REAGENTS

(a) *Soln A*.—Dissolve in  $\text{H}_2\text{O}$  180 g of  $\text{NH}_4\text{H}_2\text{PO}_4$ , 72 g of  $(\text{NH}_4)_2\text{HPO}_4$ , 0.2 g of nicotinic acid, and 0.004 g of pyridoxine hydrochloride. Add 200 ml of acid-hydrolyzed casein soln prepared in following manner and make to volume of 1000 ml with  $\text{H}_2\text{O}$ :

Mix 100 g of vitamin-free casein with 500 ml of constant-boiling HCl (ca 20% HCl) and reflux 8 hours. Remove the HCl from mixture by distillation under reduced pressure until thick sirup remains. Dissolve the sirup in  $\text{H}_2\text{O}$  and concentrate again in same manner. Redissolve resulting sirup in  $\text{H}_2\text{O}$ , adjust to pH of 3.0 with normal NaOH, and add sufficient  $\text{H}_2\text{O}$  to make 950 ml. Add to soln 20 g of activated charcoal, and stir 1 hour, then filter. Repeat treatment with activated charcoal if filtrate does not appear straw colored to colorless. Adjust pH of filtrate to 6.8 and add sufficient  $\text{H}_2\text{O}$  to bring volume to 1000 ml. (Store under toluene in refrigerator.)

(b) *Soln B*.—Dissolve in  $\text{H}_2\text{O}$  the following reagents in amounts given:

	grams
Dextrose, anhydrous	200.0
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ . . . . .	7.0
$\text{KH}_2\text{PO}_4$ . . . . .	2.2
KCl . . . . .	1.7
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ . . . . .	0.5
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ . . . . .	0.01
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ . . . . .	0.01

Make to volume of 1000 ml with  $\text{H}_2\text{O}$ .

Heat Solns A and B 30 min. at  $100^\circ$  or 15 min. at  $121^\circ$  on 3 successive days. (Solns may be heated in small portions to reduce possibility of contamination during use.) Store at room temp. until opened for use, and thereafter in refrigerator.

(c) *Yeast suspension*.—Weigh 10.0 g of fresh baker's yeast (high vitamin B, or other special yeasts are not suitable) and grind with small amount of  $\text{H}_2\text{O}$  in mortar to make smooth, creamy suspension. Add  $\text{H}_2\text{O}$  to volume of 200 ml. Prepare this suspension immediately before use.

(d) *Acid-starch-iodide indicator*.—Mix on spot plate 1 drop of each of following solns: 5% KI, 0.2% soluble starch, and 50.0%  $\text{H}_2\text{SO}_4$ .

(e) *Hydrogen peroxide soln*.—3%.

(f) *Sulfuric acid*.—0.15 N.

(g) *Sodium hydroxide soln*.—10%.

(h) *Thiamine hydrochloride stock soln*.—Weigh accurately 20–25 mg of U.S.P. Thiamine Hydrochloride Reference Standard that has been kept in desiccator over  $\text{P}_2\text{O}_5$  at least 16 hours. Since the Reference Standard is hygroscopic, take precautions to avoid absorption of moisture. Dissolve in 20% alcohol adjusted to pH 3.5–4.3 with HCl, and make to volume of 1000 ml. Store in cool place in well closed, light-resistant container.

(i) *Thiamine standard soln*.—From portion of the stock soln that has been warmed to room temp., transfer to 500 ml volumetric flask an aliquot containing



exactly 100 micrograms of thiamine hydrochloride, and dilute to 500 ml with  $H_2O$  adjusted to pH 3.5–4.3 with HCl. Each 5 ml of this soln contains 1 microgram of thiamine hydrochloride. Prepare soln on day it is used.

**36.28****APPARATUS**

Apparatus consists of thermostatically-controlled  $H_2O$  bath, shaking device, and series of reaction bottles attached to gasometers. Such apparatus is readily assembled. (Complete assembly is manufactured by American Instrument Company.) Maintain water bath thruout assay period at  $30\text{--}31^\circ \pm 0.1^\circ$ . Suitable gasometer fluid is 10%  $CaCl_2$  soln slightly colored with small amount of  $CuCl_2$ .

**36.29****PREPARATION OF SAMPLE AND BLANK**

(a) *Assay soln.*—In Erlenmeyer flask of suitable size, place accurately weighed amount of sample, estimated to contain 5 micrograms or more of thiamine, with an amount of 0.15 *N*  $H_2SO_4$  such that ratio of weight of solvent to weight of sample is at least 10 to 1. Digest 30 min. in boiling water bath or steam sterilizer, or 15 min. in autoclave at 15 lbs. pressure. Add more acid if necessary to keep mixture acid during digestion. Cool flask, adjust contents to pH of 6.0–7.0 by addition of the NaOH soln with constant stirring, and dilute with  $H_2O$  to such volume that 5–25 ml aliquot is estimated to contain 1.5 micrograms of thiamine.

(b) *Sulfite blank soln.*—To amount of sample equal to that used in preparation of assay soln add volume of 0.6%  $NaHSO_3$  soln equal to volume of 0.15 *N*  $H_2SO_4$  used in preparation of assay soln. After adjustment to pH of 5.2, digest this mixture in same manner as in (a). Cool flask and destroy excess sulfite by titration with the  $H_2O_2$  soln, using acid-starch-iodide as external indicator. (Change in color of indicator from pink to blue indicates complete destruction of sulfite.) Adjust pH of soln to 6.0–7.0 with the NaOH soln and dilute with  $H_2O$  to volume equal to that of assay soln.

**36.30****DETERMINATION**

To each of 6 reaction bottles (4 oz.) add 2.5 ml of Soln A and 7.5 ml of Soln B. To bottle No. 1 add volume of assay soln estimated to contain 1.5 micrograms of thiamine. To bottle No. 2 add (1) volume of sulfite blank soln equal to volume of assay soln used in bottle No. 1, and (2) 5 ml (1 microgram) of the thiamine standard soln, 36.27 (i). To bottles 3 and 4 add 5 ml, and to bottles 5 and 6 add 10 ml, of the thiamine standard soln. Adjust volume in each bottle to 40 ml with  $H_2O$ . Finally add 10 ml of the yeast suspension to each bottle as rapidly as possible, completing the addition within 3 min. Immediately place reaction bottles in fermentometer, connect to gasometers, and start shaking without delay. After 3 min. make reading of initial gas volume in each gasometer. (Stop shaking and equalize pressure while gasometer readings are made.) Continue shaking 3–3½ hours, then take final reading of gas volume in each gasometer. Difference between initial and final readings represents volume of gas evolved from each reaction bottle. Thruout fermentation period maintain all reaction bottles under uniform conditions of temp., pressure, and speed of shaking and take precaution to prevent leaking of gas from gasometers.

**36.31****CALCULATION OF RESULTS**

Data to be used in calculating results are valid only if they meet the following requirements:

(a) In duplicate bottles containing thiamine standard solns, variation in gas evolved is not greater than 3 ml.

(b) Difference in volumes of gas evolved from reaction bottles containing 1 microgram of reference thiamine and from those containing 2 micrograms of reference thiamine is not less than 28 ml.

(c) Volume of gas evolved from reaction bottle containing assay soln is not less than amount produced in bottle containing 1 microgram of reference thiamine standard and not greater than amount produced in bottle containing 2 micrograms of reference thiamine.

Make calculations in terms of micrograms of thiamine/reaction bottle for total fermentation activity of assay soln (bottle No. 1) and residual fermentation activity (bottle No. 2). Obtain true thiamine content of assay soln by difference. Convenient formulas for this purpose are as follows:

$$\text{I. Total fermentation activity} = 1 + \frac{X_1 - S_1}{S_2 - S_1};$$

$$\text{II. Residual fermentation activity} = \frac{X_2 - S_1}{S_2 - S_1}; \text{ and}$$

III. True thiamine = I - II, where

$X_1$  = ml of gas evolved from reaction bottle containing assay soln.

$X_2$  = ml of gas evolved from reaction bottle containing sulfite blank soln.

$S_1$  = ml of gas evolved from reaction bottle containing 1 microgram of thiamine standard.

$S_2$  = ml of gas evolved from reaction bottle containing 2 micrograms of thiamine.

This calculation is based on fact that in this range increase in fermentation response to added thiamine is a linear function.

Calculate thiamine content of sample from true thiamine content of assay soln and express in milligrams of thiamine/100 g.

For those samples in which residual fermentation activity is such that amount of gas evolved falls beyond required range, it is necessary to omit addition of thiamine standard soln to reaction bottle. Such omission must be taken into account in calculating residual fermentation activity.

#### RIBOFLAVIN (VITAMIN B<sub>2</sub>) (4)

(Applicable to yeast, dried skim milk, and alfalfa)

##### *Microbiological Method—Tentative*

#### 36.32

##### REAGENTS

(a) *Yeast extract*.—Dissolve 2 g of yeast extract (Difco Bacto yeast extract has been found to be satisfactory) in H<sub>2</sub>O and dilute to 100 ml.

(b) *Agar*.—Difco Bacto agar has been found to be satisfactory.

(c) *Yeast supplement*.—Dissolve 50 g of the yeast extract in 250 ml of H<sub>2</sub>O. Add 75 g of basic Pb acetate (Horne's sugar reagent) dissolved in 250 ml of H<sub>2</sub>O. Centrifuge off precipitate, add NH<sub>4</sub>OH to filtrate to a pH of ca 10.0, and filter off precipitate. Add acetic acid to filtrate until slightly acid, then pass in H<sub>2</sub>S until excess Pb is precipitated. Filter, and dilute filtrate to 500 ml. 1 ml of this soln = 100 mg of yeast extract. Add ca 5 ml of toluene and store in refrigerator.

(d) *Peptone, photolyzed*.—Dissolve 20 g of peptone (Difco Bacto peptone has been found to be satisfactory) in 125 ml of H<sub>2</sub>O. Dissolve 10 g of NaOH in 125 ml of H<sub>2</sub>O. Mix 2 solns, place mixture in 9" crystallizing dish, and expose to light from 100-watt bulb with reflector at distance of ca 1 ft. for 6–10 hours at temp. not exceeding 25°.

Then allow mixture to stand 18–14 hours (24 hours in all). Neutralize with acetic acid. Add 3.5 g of anhydrous  $\text{NaC}_2\text{H}_3\text{O}_2$  and dilute to 400 ml. Add ca 5 ml of toluene and store in refrigerator.

(e) *Salt soln A.*—Dissolve 25 g of  $\text{K}_2\text{HPO}_4$  and 25 g of  $\text{KH}_2\text{PO}_4$  in  $\text{H}_2\text{O}$  and make to 250 ml.

(f) *Salt soln B.*—Dissolve 10 g of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 g of  $\text{NaCl}$ , 0.5 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , and 0.5 g of  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ , and make to 250 ml.

(g) *Cystine soln.*—Dissolve 0.5 g of cystine in ca 10 ml of 10%  $\text{HCl}$  and dilute to 500 ml with  $\text{H}_2\text{O}$ . Add 5 ml of toluene and store in refrigerator.

(h) *Dextrose.*—Anhydrous reagent grade.

(i) *Pure culture of Lactobacillus casei.*—This culture may be obtained from American Type Culture Collection, Georgetown University Medical School, 3900 Reservoir Rd., Washington, D. C. Ask for *Lactobacillus casei* No. 7469. Cost of one culture is \$2.00 plus 35¢ to cover cost of packing and postage.

(j) *Riboflavin solns.*—Store in dark bottles in refrigerator. Add 5 ml of toluene. Protect from light.

*Stock soln.*—Weigh 50 mg of U.S.P. Riboflavin Reference Standard, dissolve in  $\text{H}_2\text{O}$ , and dilute to 500 ml with 0.02 N acetic acid (1.2 g of acetic acid to 1000 ml of  $\text{H}_2\text{O}$ ). (This soln contains 50 micrograms of riboflavin per ml.)

*Standard soln.*—Dilute 1 ml of the stock soln in  $\text{H}_2\text{O}$  to 1000 ml. (This soln contains 0.1 microgram of riboflavin/ml.) Prepare fresh for each assay.

(k) *Sodium chloride soln.*—Dissolve 9 g of  $\text{NaCl}$  in  $\text{H}_2\text{O}$  and make to 1000 ml. Sterilize this soln when needed by placing 10 ml in test tubes and autoclaving at 15 lbs. pressure for 20 min.

(l) *Basal medium stock soln.*—Mix 100 ml of the photolyzed peptone, 100 ml of the cystine soln, 10 ml of the yeast supplement, 5 ml of salt soln A, and 5 ml of salt soln B. In the mixture of these solns dissolve 30 g of the dextrose.

Test pH and adjust to 6.6–6.8 if necessary by small additions of 0.5%  $\text{NaOH}$  soln or  $\text{HCl}$  (1+10). (Use bromothymol blue as indicator.) Dilute to 500 ml.

### 36.33

#### PREPARATION OF INOCULUM

(a) *Agar culture medium.*—Dissolve 1.0 g of the dextrose in 100 ml of the yeast extract and add 1.5 g of the agar. Heat mixture in autoclave or in water bath until agar is dissolved. Dilute to 100 ml and mix well. Place 10 ml portions in test tubes and plug with cotton. Sterilize in autoclave at pressure of 15 lbs. for 20 min. Allow tubes to cool in upright position.

(b) *Stock cultures.*—Make stab cultures into 3 or more of the agar tubes from the original pure culture of *Lactobacillus casei*. Incubate at 37° for 24 hours and store in refrigerator until needed. Keep one culture (especially if future work is to be done) as reserve stock culture and do not disturb except to make new stock cultures from it at end of 1 month. (Other stock tubes are to be used for analysis and are good for 1 month only. If work is to be done after that time, make new stock cultures from reserve stock culture.) Discard cultures when 1 month old.

(c) *Sodium chloride inoculum.*—Pipet 5 ml of basal medium stock soln into 3 test tubes (16×150 mm to 20×150 mm). To each tube add 5 ml of  $\text{H}_2\text{O}$  containing 1 microgram of riboflavin (final volume 10 ml). Plug tubes with cotton and sterilize by autoclaving at 15 lbs. pressure for 15 min. Avoid exposing tubes to light at any time after riboflavin is added. After tubes are cool, inoculate with stabs from one of stock cultures. Incubate 24 hours at 37°. Centrifuge cells aseptically. Resuspend cells from one of tubes in 10 ml of sterile 0.9%  $\text{NaCl}$  soln. This culture is to be used as the inoculum.

## 36.34

## PREPARATION OF SAMPLE

(a) *Yeast or other materials containing over 20 p.p.m. of riboflavin.*—To 2 g add 200 ml of  $H_2O$ , mix well, and autoclave at  $120^\circ$  for 15 min. Centrifuge off insoluble matter and wash twice with 20 ml of  $H_2O$ . Combine extracts and dilute to 1000 ml in graduated flask. 1 ml of this extract = 2 mg of sample. Avoid exposure to light at all times.

(b) *Skim milk or other samples containing 10–20 p.p.m. of riboflavin.*—To 3 g add 150 ml of 0.1 *N* HCl. Mix well and autoclave 15 min. at  $120^\circ$ . Adjust pH to 6.6–6.8 with 0.1 *N* NaOH (bromothymol blue indicator) and dilute to 500 ml. Assay this suspension directly. 1 ml = 6 mg of sample. Avoid exposure to light.

(c) *Alfalfa.*—To 3 g add 150 ml of 0.1 *N* HCl. Mix well and autoclave 15 min. at 15 lbs. pressure. Centrifuge off insoluble matter and wash twice with 20 ml of  $H_2O$ . To the insoluble matter add 150 ml of normal HCl and autoclave 15 min. Centrifuge, and wash as directed above. Combine extracts and adjust pH to 6.6–6.8 with 1.0 *N* NaOH, using either bromothymol blue indicator or pH meter. Dilute to 500 ml. 1 mg of this extract = 6 mg of sample.

## 36.35

## DETERMINATION

With each set of assays use series of tubes containing graded levels of riboflavin. Use duplicate tubes, each containing 0.0, 0.05, 0.075, 0.1, 0.15, 0.2, and single tubes, each containing 0.3 and 0.5 microgram of riboflavin. With microburet, measure 0.0, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, and 5.0 ml of the standard riboflavin soln into test tubes (specified above) and pipet in 5 ml of the basal medium stock soln. Add enough  $H_2O$  to make final volume 10 ml.

Use duplicate tubes for samples to be analyzed containing equivalent of 0.5, 1, 2, 3, 4, and 5.0 mg of yeast or 1.5, 3, 6, 9, 12, and 15 mg of dried skim milk or alfalfa. Measure 0.25, 0.5, 1.0, 1.5, 2, and 2.5 ml of yeast or alfalfa extracts or of skim milk suspension into test tubes and pipet in 5 ml of the basal medium stock soln. Add enough  $H_2O$  to make final volume 10 ml.

Plug tubes with cotton and sterilize in autoclave at 15 lbs. pressure 15 min. Allow to cool, and inoculate each with 1 drop of the NaCl inoculum. Incubate at  $37^\circ$  for 3 days. Transfer contents to 125 ml Erlenmeyer flasks, wash tubes with 10–20 ml of  $H_2O$ , and titrate from microburet to pH of 7.0 with 0.1 *N* NaOH, using bromothymol blue indicator.

Plot micrograms of riboflavin against ml of 0.1 *N* NaOH used in titration of the standard riboflavin soln. If blank is 1 ml or over, ingredients of basal medium stock soln contain riboflavin and results should not be used. Repeat with reagents more rigorously freed from riboflavin.

From volume of 0.1 *N* NaOH used in titration for each level of yeast, dried skim milk, or alfalfa, locate on graph corresponding micrograms of riboflavin. These values should be 0.05–0.25 micrograms, otherwise they are not reliable. If 3 of the values do not fall within this range, repeat analysis with amount of sample calculated to bring values within this range. Calculate p.p.m. (micrograms/gram) for each value in range specified. (Results from both determinations should agree within 20%). Average for final result.

*Fluorometric Method—Tentative*

## 36.36

## REAGENTS

(a) *Sulfuric acid.*—Approximately 0.25 *N*. Dilute 5 ml of  $H_2SO_4$  to 800 ml with  $H_2O$ .

(b) *Trisodium phosphate soln.*—Dissolve 65 g of  $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$  in 1 liter of  $\text{H}_2\text{O}$ .

(c) *Acid-acetone soln.*—Add 3 volumes of commercial acetone to 1 volume of normal  $\text{H}_2\text{SO}_4$  (5 ml of  $\text{H}_2\text{SO}_4$  in 200 ml of  $\text{H}_2\text{O}$ ). (This reagent is used only if products contain casein.)

(d) *Sodium hydrosulfite soln.*—Dissolve 1 g of  $\text{Na}_2\text{S}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$  and 1 g of  $\text{NaHCO}_3$  in 20 ml of ice-cold  $\text{H}_2\text{O}$  and keep in ice bath. (This soln is stable ca 4 hours.)

(e) *Stannous chloride.*—Dissolve 10 g of  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  in 25 ml of  $\text{HCl}$  and store in brown glass-stoppered bottle. For determination dilute 1 ml of above stock soln with 200 ml of  $\text{H}_2\text{O}$ . Make fresh preparation of the dilute soln daily.

(f) *Standard riboflavin solns.*—Store in dark bottles in refrigerator. Rigorously protect from light.

(1) *Soln A.*—Weigh 20 mg of U.S.P. Riboflavin Reference Standard, dissolve, add a few drops of acetic acid, and dilute to 500 ml with  $\text{H}_2\text{O}$ . This soln contains 40 p.p.m. of riboflavin.

(2) *Soln B.*—Pipet 25 ml of Soln A into 100 ml flask and dilute to volume. This soln contains 10 p.p.m. of riboflavin.

(g) *Standard quinine sulfate solns.*—Used to regulate intensity of activating light of a fluorometer. (They cannot be used for visual comparisons of riboflavin. Sodium fluorescein or standard glass cube may be substituted for quinine sulfate.)

(1) *Soln A.*—Weigh 40 mg of quinine sulfate, dissolve in ca 20 ml of 0.1  $N$   $\text{H}_2\text{SO}_4$ , and dilute to 200 ml with the acid.

(2) *Soln B.*—Pipet 10 ml of Soln A into 100 ml graduated flask and make to volume with 0.1  $N$   $\text{H}_2\text{SO}_4$ .

(3) *Soln C.*—Pipet 10 ml of Soln B into 1000 ml graduated flask and make to volume with 0.1  $N$   $\text{H}_2\text{SO}_4$ . Soln C contains 0.2 p.p.m. of quinine sulfate.

### 36.37

#### CALIBRATION OF FLUOROMETER

(The directions given are for calibration of fluorometer manufactured by Pfaltz and Bauer, Inc., New York. Other types of instruments may be used. Such instruments may not have same sensitivity as the Pfaltz and Bauer instrument and therefore riboflavin solns of different concentrations may be needed.)

Pipet 20 ml of standard riboflavin soln B into 200 ml graduated flask and dilute to volume (*Soln C*). Dilute 40 ml of Soln C to 50 ml (*Soln D*). Dilute 10 ml of Soln B to 200 ml (*Soln E*). Dilute 40 ml of Soln E to 50 ml (*Soln F*), 30 ml of Soln E to 50 ml (*Soln G*), 20 ml of Soln E to 50 ml (*Soln H*), 10 ml of Soln E to 50 ml (*Soln I*), 5 ml of Soln E to 50 ml (*Soln J*), and 0.0 ml of Soln E to 50 ml (*Blank*). Soln C contains 1.0 p.p.m. riboflavin, Soln D 0.8, Soln E 0.5, Soln F 0.4, Soln G 0.3, Soln H 0.2, Soln I 0.1, and Soln J 0.05.

Place quinine sulfate Soln C in cell of fluorometer and adjust iris diaphragm of instrument to give galvanometer deflection of 65 mm. Remove the quinine sulfate soln and obtain galvanometer deflections for riboflavin solutions C, D, E, F, G, H, I, J, and blank. Also obtain galvanometer deflections for above riboflavin solns for quinine sulfate deflections of 50.0, 33.0, and 18.2. Plot curve of concentration of riboflavin against galvanometer deflection for each deflection of quinine sulfate. Subtract reading for blank from each value. Place all curves on same graph. By above procedure a wide range of concentrations of riboflavin can be read on fluorometer.

### 36.38

#### PREPARATION OF SAMPLE

(a) *Materials that do not contain casein.*—Avoid exposure to light as much as possible. Weigh 5 g into 300 ml Erlenmeyer flask and pipet in 50 ml of the 0.25  $N$   $\text{H}_2\text{SO}_4$ . Mix thoroly and break up lumps. Boil gently under reflux condenser 1 hour.

Allow sample to come to room temp. and disconnect condenser. Bring pH to 7.0–7.5 with the  $\text{Na}_3\text{PO}_4$  soln. Use phenol red or other suitable indicator. (30 ml of the  $\text{Na}_3\text{PO}_4$  soln may be added before soln is tested for pH.) Use only 1 drop of soln for each pH test. Transfer soln to 100 ml graduated flask and make to volume. Allow mixture to stand 30 min. and filter thru fluted filter. Pipet 5 ml aliquot into 200 ml volumetric flask and dilute to ca 175 ml with  $\text{H}_2\text{O}$ . Add 2 ml of the  $\text{Na}_2\text{S}_2\text{O}_4$  soln and 2 ml of the  $\text{SnCl}_2$  soln. Make to volume, mix well, and allow to stand 10 min. Pour into 1 liter Erlenmeyer flask and shake vigorously 5 min. with access to air. Pipet 2 aliquots of 50 ml each into 100 ml Erlenmeyer flasks. Pour remainder into 100 ml flask but do not measure.

(b) *Materials containing casein*.—Proceed as directed in (a) except to use the acid-acetone soln for extraction instead of  $\text{H}_2\text{SO}_4$ . Also use 50 ml aliquot for reduction with  $\text{Na}_2\text{S}_2\text{O}_4$  and  $\text{SnCl}_2$ .

## 36.39

## DETERMINATION

Place portion of the standard quinine sulfate soln in cell of fluorometer and adjust iris diaphragm to give galvanometer deflection of 65.0, 50.0, 33.0, or 18.2, according to concentration of riboflavin expected in unknown soln. Place part of unknown soln in fluorometer, obtain galvanometer deflection, and calculate to riboflavin from calibration curve (A). To one of 50 ml portions of unknown add with pipet 1 ml of riboflavin Soln B, 36.36, (f)(2), and obtain galvanometer deflection. Convert to riboflavin (B). To remaining 50 ml aliquot of unknown add 1 ml of the  $\text{Na}_2\text{S}_2\text{O}_4$  soln, mix well, and read deflection. Convert to riboflavin (C). To 50 ml of  $\text{H}_2\text{O}$  add 1 ml of riboflavin Soln B and obtain deflection. Subtract deflection given by  $\text{H}_2\text{O}$  from reading and convert to riboflavin (D).

## 36.40

## CALCULATION

$$\left( A - 1.02C \right) \left( \frac{1.02D}{1.02B - A} \right) F = \text{p.p.m. of riboflavin, where}$$

A, B, C, and D are specified above, and F is dilution factor. No correction is necessary for deflection caused by reagents in unknown. It is eliminated in above calculation.

## NICOTINIC ACID (NIACIN) OR NICOTINAMIDE (NIACINAMIDE)

*Microbiological Method—Tentative*

## 36.41

## REAGENTS

(a) *Acid-hydrolyzed casein soln*.—See 36.27(a).

(b) *Cystine soln*.—Dissolve 1 g of l-cystine in 20 ml of 10% HCl and add sufficient  $\text{H}_2\text{O}$  to make 1000 ml. Store under toluene in refrigerator not below 10°.

(c) *Tryptophane soln*.—Dissolve 1 g of l-tryptophane in 5–6 ml of 20% HCl and add sufficient  $\text{H}_2\text{O}$  to make 1000 ml. Store under toluene in refrigerator.

(d) *Adenine-guanine-uracil soln*.—Dissolve 0.1 g each of adenine sulfate, guanine hydrochloride, and uracil with aid of heat in 5 ml of 20% HCl, and add sufficient  $\text{H}_2\text{O}$  to make 100 ml. Store in refrigerator.

(e) *Thiamine hydrochloride soln*.—Prepare soln containing 0.1 mg/ml by dissolving crystalline thiamine hydrochloride in 25% alcohol adjusted to pH 3 with HCl. Store in refrigerator.

(f) *Calcium pantothenate soln*.—Prepare soln containing 0.1 mg/ml by dissolving crystalline calcium pantothenate in neutral 25% alcohol. Store in refrigerator.

(g) *Pyridoxine hydrochloride soln.*—Prepare soln containing 0.1 mg/ml by dissolving crystalline pyridoxine hydrochloride in 25% alcohol. Store in refrigerator.

(h) *p-Aminobenzoic acid soln.*—Prepare a soln containing 0.1 mg/ml by dissolving crystalline p-aminobenzoic acid in 25% alcohol. Store in refrigerator.

(i) *Riboflavin soln.*—Prepare soln containing 0.1 mg/ml by dissolving crystalline riboflavin in 0.02 *N* acetic acid. Store soln, protected from light, in refrigerator.

(j) *Biotin soln.*—Prepare a soln containing 0.1 microgram/ml by dissolving crystalline biotin (free acid) in 50% alcohol. Store in refrigerator.

(k) *Salt soln A.*—Dissolve 25 g of  $\text{KH}_2\text{PO}_4$  and 25 g of  $\text{K}_2\text{HPO}_4$  in sufficient  $\text{H}_2\text{O}$  to make 250 ml of soln.

(l) *Salt soln B.*—Dissolve 10 g of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 g of NaCl, 0.5 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , and 0.5 g of  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$  in sufficient  $\text{H}_2\text{O}$  to make 250 ml.

(m) *Standard nicotinic acid soln.*—Accurately weigh 50 mg of U.S.P. Nicotinic Acid Reference Standard and add sufficient alcohol to make 500 ml. Store this stock soln in refrigerator. Prepare the standard soln by diluting 1 ml of the stock soln, which has been warmed to room temp., with sufficient  $\text{H}_2\text{O}$  to make 1000 ml. This soln contains 0.1 microgram of the reference standard in each ml of soln. Prepare fresh for each assay.

(n) *Basal medium stock soln.*—(Sufficient for 50 tubes.)

	ml
Acid-hydrolyzed casein soln . . . . .	25
Tryptophane soln . . . . .	50
Cystine soln . . . . .	100
Adenine-guanine-uracil soln . . . . .	5
Thiamine hydrochloride soln . . . . .	0.5
Calcium pantothenate soln . . . . .	0.5
Pyridoxine hydrochloride soln . . . . .	1
Riboflavin soln . . . . .	1
p-Aminobenzoic acid soln . . . . .	0.5
Biotin soln . . . . .	2
Salt soln A . . . . .	2.5
Salt soln B . . . . .	2.5
	grams
Anhydrous dextrose . . . . .	10
Sodium acetate . . . . .	5

Mix ingredients, adjust soln to pH 6.8, and add sufficient  $\text{H}_2\text{O}$  to make 250 ml.

### 36.42

#### PREPARATION OF INOCULUM

(a) *Stock culture of Lactobacillus arabinosus 17-5.*—To 5 ml of yeast extract soln in 95 ml of  $\text{H}_2\text{O}$ , add 1 g of anhydrous dextrose and 1.5 g of agar, and heat mixture on steam bath until agar has dissolved. Add ca 10 ml portions of the hot soln to test tubes, plug tubes with non-absorbent cotton, sterilize in autoclave at 15 lbs. pressure (121.5°) for 20 min., and allow to cool in upright position. Prepare stab cultures, using pure culture of *Lactobacillus arabinosus 17-5* (this culture may be obtained as culture No. 8014 from American Type Culture collection, Georgetown University Medical School, Washington, D. C.), incubate 16–24 hours at any selected temp. between 30° and 37° held constant to within  $\pm 0.5^\circ$ , and finally store in refrigerator. Prepare fresh stab of stock culture every week and do not use for inoculum if culture is more than 2 weeks old.

(b) *Culture medium.*—To each of series of tubes containing 5 ml of the basal medium stock soln add 5 ml of  $\text{H}_2\text{O}$  containing 2 micrograms of nicotinic acid. Sterilize autoclave at 15 lbs. pressure (121.5°) for 20 min.

(c) *Inoculum*.—Transfer cells from stock culture of *Lactobacillus arabinosus* 17-5 to sterile tube containing 10 ml of culture medium. Incubate this culture 16-24 hours at any selected temp. between 30° and 37° held constant to within  $\pm 0.5^\circ$ . Under aseptic conditions centrifuge the culture and decant supernatant liquid. Prepare inoculum by suspending cells from culture in 10 ml of sterile isotonic NaCl soln. If assays are to be made on each of several successive days, inoculum may be prepared by successive daily transfers in culture medium for period not exceeding 1 week.

**36.43**

## PREPARATION OF SAMPLE

Place sufficient material, accurately weighed, to represent 0.02-0.1 mg of nicotinic acid, in 300 ml flask, add 100 ml of normal  $\text{H}_2\text{SO}_4$ , and mix thoroly. Heat in autoclave at 15 lbs. pressure (121.5°) for 30 min., cool, add normal NaOH to pH 6.8, and dilute with  $\text{H}_2\text{O}$  to volume such that 1 ml contains ca 0.1 microgram of nicotinic acid.

**36.44**

## DETERMINATION

Prepare standard nicotinic acid tubes as follows: To duplicate tubes 16×150 mm in size, add 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 ml, respectively, of the standard nicotinic acid soln. To each of these tubes add 5 ml of basal medium stock soln and sufficient  $\text{H}_2\text{O}$  to bring the volume in each tube to 10 ml.

Prepare tubes containing material to be assayed as follows. To duplicate tubes add, respectively, 1.0, 2.0, 3.0, and 4.0 ml of sample soln, 36.43. To each of these tubes add 5 ml of the basal medium stock soln and sufficient  $\text{H}_2\text{O}$  to bring volume in each tube to 10 ml.

After thoro mixing, plug tubes of two series mentioned above with non-absorbent cotton, and autoclave at 15 lbs. pressure (121.5°) for 20 min. Cool, aseptically inoculate each tube with 1 drop of inoculum, and incubate 72 hours at any selected temp. between 30° and 37°, held constant to within  $\pm 0.5^\circ$ . Contamination of assay tubes with any organisms other than *Lactobacillus arabinosus* invalidates the assay.

Transfer contents of each tube to suitable container, using ca same quantity of  $\text{H}_2\text{O}$  in each instance for rinsing. Titrate contents of each flask with 0.1 *N* NaOH, using bromothymol blue indicator, or to pH of 6.8 measured electrometrically.

**36.45**

## CALCULATION

Prepare standard curve of the nicotinic acid standard titrations by plotting average of titration values expressed in ml of 0.1 *N* NaOH for each level of nicotinic acid standard soln used, against micrograms of nicotinic acid contained in the respective tubes. From this standard curve determine by interpolation the nicotinic acid content of the test soln in each duplicate set of tubes. Discard any values that show more than 0.4 or less than 0.05 microgram of nicotinic acid in each tube. Calculate nicotinic acid content in each ml of test soln for each of the duplicate sets of tubes, and that of the test material, from average of values obtained from not less than 3 sets of these tubes that do not vary by more than  $\pm 10\%$  from average. If titration values of two or more of the duplicate sets of tubes containing the test soln fall below titration values of the nicotinic acid standard tubes containing 0.05-0.4 mg of nicotinic acid, nicotinic acid content of test soln is too low to permit calculation of nicotinic acid content of test material. Titration values exceeding 2 ml for tubes of the standard nicotinic acid soln series containing 0.0 ml of the soln indicate presence of excessive amount of nicotinic acid in basal medium stock soln and invalidate the assay.



## 36.46

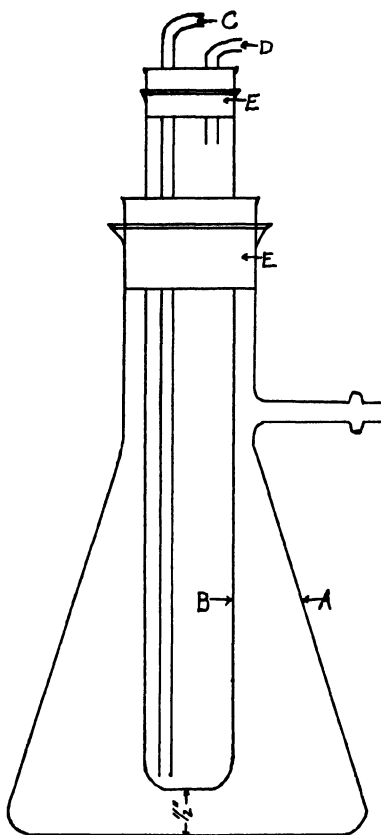
*Sublimation Method (5)—Tentative*

FIG. 57.—FITTED FLASK FOR DETERMINATION OF NICOTINIC ACID

(Applicable to tablets and ampuls of nicotinic acid)

(a) *Tablets*.—Weigh by difference ca 0.25 g of powdered sample into 500 ml suction flask (Fig. 57). Fit flask with inside condenser consisting of 10" test tube, held in neck of flask by rubber stopper, and extending to within  $\frac{1}{2}$ " of bottom of flask. Test tube should have clearance of ca  $\frac{1}{4}$ " on all sides in neck of flask. Connect flask thru two way stopcock to aspirator or vacuum line. Circulate  $H_2O$  thru condenser by means of glass tube extending to bottom of test tube. Immerse flask in glycerin bath up to side arm and hold in this position by means of clamp. Raise temp. of bath to ca  $160^\circ$ , stirring occasionally, and hold at this temp. 1 hour. Remove burner and raise flask out of bath. Cool, and turn off vacuum. Carefully fasten condenser with clamp to ring stand to avoid shaking off sublimate. Break vacuum and cautiously remove flask. Wash sublimate from tube with stream of hot absolute alcohol into weighed beaker, evaporate alcohol on steam bath in current of air, and dry 20 min. at  $100^\circ$ . Cool in desiccator and weigh.

(b) *Ampuls*.—With pipet measure 5 ml of sample into 500 ml suction flask and evaporate  $H_2O$  on steam bath with aid of current of air directed into flask. Dry residue in oven at  $100^\circ$  30 min. and proceed as directed under (a), beginning "Fit flask with inside condenser."

## VITAMIN C

## ASCORBIC ACID (6)—OFFICIAL, FIRST ACTION

(Applicable to orange, grapefruit, lemon, lime, and tomato juice provided the juices do not contain ferrous  $Fe$ ,  $SO_2$ , sulfite, or thiosulfate)

## 36.47

## REAGENTS

(a) *Metaphosphoric acid-acetic acid stabilizing extracting soln.*—Dissolve with shaking 15 g of freshly pulverized stick  $HPO_3$  in 40 ml of acetic acid and 200 ml of  $H_2O$ , dilute to ca 500 ml, and filter rapidly thru fluted filter into glass-stoppered bottle. (The  $HPO_3$  slowly changes to  $H_3PO_4$ , but if stored in refrigerator it remains satisfactory for 7–10 days.)

(b) *Standard ascorbic acid*.—Use U.S.P. Reference l-Ascorbic Acid (obtainable

from U.S.P. Revision Committee, 43rd St. and Woodland Ave., Philadelphia, Pa.). Keep cool, dry, and out of light.

(c) *Standard indophenol soln.*—Dissolve 0.05 g of reagent-grade Na 2,6-dichlorobenzenoneindophenol, which has been stored in desiccator over soda lime, in 50 ml of H<sub>2</sub>O to which has been added 42 mg of NaHCO<sub>3</sub>; shake vigorously, and when dye has dissolved dilute to 200 ml with H<sub>2</sub>O. Filter thru fluted filter into amber glass-stoppered bottle. Keep stoppered, out of direct sunlight, and store in refrigerator. (Decomposition products that make end point indistinct occur in some batches of dry indophenol and also develop with time in the stock soln. Add 5.0 ml of extracting agent containing excess ascorbic acid to 15 ml of the dye reagent. If the reduced soln is not practically colorless, discard and prepare new stock soln. If dry dye is at fault, obtain new specimen.)

Weigh accurately ( $\pm 0.1$  mg) ca 0.1 g of the standard ascorbic acid, transfer to 100 ml glass-stoppered volumetric flask, and bring to mark (room temp.) with the HPO<sub>3</sub>-acetic acid reagent. Standardize the indophenol soln at once as follows: Transfer three 2.0 ml aliquots of the ascorbic acid soln to each of three 50 ml Erlenmeyer flasks containing 5.0 ml of the HPO<sub>3</sub>-acetic acid reagent. Titrate rapidly with the indophenol soln from 50 ml buret until a light but distinct rose-pink color persists for at least 5 seconds. (Each titration should require ca 15 ml of the indophenol soln, and titrations should check within 0.1 ml.) In like manner titrate 3 blanks composed of 7.0 ml of the HPO<sub>3</sub>-acetic acid reagent plus a volume of H<sub>2</sub>O ca equivalent to volume of indophenol soln used in direct titrations. After subtracting average blanks (usually ca 0.1 ml) from standardization titrations, calculate and express concn of indophenol soln as mg of ascorbic acid equivalent to 1.0 ml of reagent. Standardize the indophenol soln each day of use with freshly prepared standard ascorbic acid soln.

### 36.48

#### PREPARATION OF SAMPLE AND DETERMINATION

Prepare juices as directed under 26.2. Add aliquots of at least 100 ml of prepared juice to equal volumes of the HPO<sub>3</sub>-acetic acid reagent. Mix, and filter rapidly thru loose-texture folded filter (Eaton-Dikeman paper No. 195, folded filter 18½ cm size, or equivalent). Titrate 10 ml aliquots as directed for standardization of the indophenol soln, and make blank determinations for corrections of titrations as described under 36.47(c), using proper volumes of acid reagent and H<sub>2</sub>O. Express ascorbic acid as mg/100 ml of original juices.

NOTE: Products containing ferrous Fe, originating from defective cans, etc., give values in excess of their actual ascorbic acid content by this method. Following is simple test to ascertain whether ferrous Fe is present to extent that invalidates test: Add 2 drops of 0.05% H<sub>2</sub>O soln of methylene blue to 10 ml of freshly prepared mixture of juice and the HPO<sub>3</sub>-acetic acid reagent. Disappearance of methylene blue color in 5–10 seconds indicates presence of interfering substances.

## VITAMIN D

### VITAMIN D IN MILK (7)—TENTATIVE

### 36.49

#### PRESERVATION OF SAMPLE

Sample of the fluid milk to be assayed must be delivered to assayer in original container immediately after collection or be stored under refrigeration in iced container until delivered. After delivery to assayer, the milk must be preserved in its homogeneous state by refrigeration at temp. of not more than 10°C (50°F) for period of not more than 10 days, or else be preserved for not more than 30 days by addition of 2 drops of 10% HCHO soln to one quart of milk in addition to refrigeration at temp. of not more than 10°C (50°F). Evaporated and reconstituted milk must be

preserved in same manner as fluid milk. A sample soured or curdled is unsuitable for assay purposes. A sample of dried milk, after being opened by the assayer, must be preserved by refrigeration at temp. of not more than 10°C (50°F).

**36.50****PRELIMINARY PERIOD**

Thruout preliminary period each rat must be raised under immediate supervision of, or according to directions specified by, the assayer. Thruout preliminary period, maintain rats on dietary regimen that provides for normal development in all respects, except to limit supply of vitamin D to such degree that rats, weighing between 40 and 60 g at age of 21–30 days, and subsisting for interval of 18–25 days on suitable rachitogenic diet, manifest evidence of severe rickets.

**36.51****DEPLETION PERIOD**

A rat is suitable for depletion period when its age does not exceed 30 days, and its body weight exceeds 44 g but not 60 g, provided it manifests no evidence of injury, or disease, or anatomical abnormality that might hinder growth and development. Thruout depletion period provide each rat with rachitogenic diet, and H<sub>2</sub>O or U.S.P. water *ad libitum*, and permit no other dietary supplement to be available.

**36.52****ASSEMBLING RATS INTO GROUPS FOR ASSAY PERIOD**

Assemble rats that are suitable for the assay period into groups. For each assay milk provide one or more assay groups. In the assay of one milk provide at least one reference group, but one reference group may be used for concurrent assay of more than one assay milk. On any one day during interval of assembling rats into groups, total number of rats assigned to make up any one group must not exceed by more than 2 the number of rats that have been assigned to make up any other group. When assembling of all groups is completed, total number of rats in each group must be the same. Assign not more than 3 rats from 1 litter to the assay group unless equal number of rats from same litter is assigned to reference group. There must be sufficient number of animals in each group to meet requirements specified under 36.56.

**36.53****ASSAY PERIOD**

A rat is suitable for assay period, provided depletion period has exceeded 18 days but not 25 days, and provided the rat manifests evidence of rickets characterized by distinctive, wobbly, rachitic gait and enlarged joints. Presence of rickets may also be established by examination of a leg bone of one member of a litter by the "line test," 36.54, or by x-ray examination of the animals selected for the assay. Keep each rat in individual cage, provided with the rachitogenic diet and H<sub>2</sub>O or U.S.P. water *ad libitum*. On any calendar day of assay period the assay and reference groups must receive a rachitogenic diet compounded from same lots of ingredients. Thruout first 6 days of assay period feed each rat in any one assay group daily a dose of the assay milk, and thruout first 6 days of assay period feed each rat in any one reference group daily a dose of reference oil, and in addition, and separate from the rachitogenic diet, an amount of ether-extracted skim milk powder equal in weight to the solids-not-fat contained in the daily dose of assay milk fed the assay group, except that the daily dose of milk or reference oil plus ether-extracted skim milk powder may be doubled on day preceding a one-day holiday. During remainder of assay period, feed neither the assay milk nor the reference oil plus ether-extracted skim milk powder. The following optional methods of feeding assay milk and reference oil plus ether-extracted skim milk powder are permissible, but both assay milk

and reference oil plus ether-extracted skim milk powder must be fed according to same method: the supplements may be fed on first day of assay period or in equal portions on first, third, and fifth days of a 7-day or 10-day assay period or on first 8 days of a 10-day assay period; the supplements may be fed admixed with quantity of the basal ration that will be consumed within the first 5 days of a 7-day assay period or within the first 8 days of a 10-day assay period. In each case, make unsupplemented basal ration available during remainder of assay period. Feed quantity of reference oil found by experience to cause extent and degree of calcification of the rachitic metaphysis equal to or greater than a condition described as positive macroscopic evidence of calcification, but less than extent and degree of calcification described as complete healing. When a vitamin D milk is to be assayed to determine whether vitamin D potency is that claimed, feed that quantity of assay milk calculated to contain same number of units of vitamin D as contained in quantity of reference oil fed. Calculate quantity of ether-extracted skim milk powder to be fed on basis of 9% solids-not-fat in whole milk. At the termination of the assay period kill each rat and examine one or more leg bones for healing of the rachitic metaphysis according to the "line test," 36.54. Reference oil may be diluted before being fed with an edible vegetable oil free from vitamins A and D. Diluted oil must be stored in dark at temp. not exceeding 10°C (50°F), for not more than 30 days. Do not feed more than 1/10 ml of the diluted oil as a daily dose. The ether-extracted skim milk powder may be fed as dry powder or it may be reconstituted with H<sub>2</sub>O to liquid skim milk. During assay period maintain all conditions of environment (particularly with reference to physiologically active radiations) as uniform as possible with respect to the assay and reference groups.

**36.54****LINE TEST**

Make line test on proximal end of a tibia or distal end of a radius or ulna. End of desired bone is removed from animal and cleaned of adhering tissue. A longitudinal median section is made thru end of the bone with clean, sharp blade to expose plane surface thru junction of epiphysis and diaphysis. In any one assay the same bone of all animals must be used and sectioned thru same plane. Rinse both sections of the bone in H<sub>2</sub>O and then immerse in 2% AgNO<sub>3</sub> soln for 1 min. Then rinse sections in H<sub>2</sub>O and expose the sectioned surfaces in H<sub>2</sub>O to daylight or other source of actinic light until calcified areas have developed clearly defined stains without marked discoloration of uncalcified areas. Record immediately extent and degree of calcification of the rachitic metaphysis of every section. Modifications of the described procedure for staining may be used, provided such modified procedures clearly differentiate between calcified and uncalcified areas. Distinguish between staining due to congestion in the rachitic metaphysis and healing as clearly indicated by presence of Ca salts stained with Ag.

**36.55****RECORDING OF DATA**

On day beginning assay period and on 7th or 10th day thereafter, depending on duration of assay period, record body weight of each rat. Keep record of quantity of rachitogenic diet consumed per rat during assay period. Assign numerical values to extent and degree of calcification of rachitic metaphyses of bones examined by line test so that it will be possible to average performance of each group.

**36.56****POTENCY OF THE ASSAY MILK**

Consider data from a reference group valid for establishing vitamin D potency of the assay milk only when two-thirds or more, but not less than 7 rats, show individ-

usually an extent and degree of calcification of the rachitic metaphysis equal to or greater than condition described as positive macroscopic evidence of calcification, but less than an extent and degree of calcification described as complete healing. When average response of assay group (in which two-thirds or more, but not less than 7 rats, show individually an extent and degree of calcification described as positive macroscopic evidence of healing) is equal to or greater than that of the reference group, the vitamin D content of milk fed during assay period is equal to or greater than the vitamin D content of reference oil fed during assay period. When average response of the assay group is less than that of reference group, the vitamin D content of milk fed during assay period is less than that of reference oil fed during assay period. Consider data from a rat valid for establishing average performance of a group only when weight of rat at termination of assay period equals or exceeds the weight of the rat on beginning day of assay period, and provided that the rat has consumed an average of not less than 4 g of rachitogenic diet daily during assay period and has consumed each prescribed dose of assay milk within 24 hours from the time it was fed. When above conditions have been met and response of assay group is equal to that of reference group, vitamin D potency of the assay milk may be calculated as follows:

## 36.57

## CALCULATION

Let  $R$  = total number of U.S.P. units of vitamin D fed each rat in reference group during assay period.

Let  $M$  = ml of assay milk fed each rat in the assay group during the assay period.

Then  $R \times \frac{946}{M}$  = U.S.P. units of vitamin D/quart. (946 = ml in 1 quart.)

## 36.58

## DEFINITIONS

The term *assay group* means a group of rats to which the assay milk (vitamin D milk) is administered during the assay period. The term *assay milk* means the milk (vitamin D milk) under examination for its vitamin potency. The term *assay period* means the interval in the life of a rat between the last day of the depletion period and the eighth or eleventh day thereafter. The term *assemble* means the procedure by which rats are selected and assigned to groups for the purpose of feeding, care, and observation. The term *daily* means each of the first 6 or 8 days of the assay period. The term *depletion period* means the interval in the life of a rat between the last day of the preliminary period and the first day of the assay period. The term *dose* means the quantity of reference oil or of assay milk or other supplement to be fed daily to a rat during an assay period. The term *feed* means make readily available to the rat or administer to the rat by mouth. The term *ground gluten* means the clean, sound product made from wheat flour by almost complete removal of starch; it must contain not more than 10% of  $H_2O$ , and, calculated on  $H_2O$ -free basis, not less than 14.2% of N, not less than 15% of N-free extract (using protein factor 5.7), and not more than 5.5% of starch (as determined by diastase method, 27.35). The term *group* means 7 or more rats maintained on same required dietary regimen during assay period. The term *preliminary period* means the interval in the life of a rat between the seventh day after birth and the first day of the depletion period. The term *reference oil* means U.S.P. Reference Cod Liver Oil distributed by the Board of Trustees of the United States Pharmacopoeial Convention. The term *rachitogenic diet* means the uniform mixture of the food material, and in the proportions named, in either of following formulas:

## 36.59

*Rachitogenic Diet No. 1*

	<i>per cent</i>
Whole yellow maize, ground . . . . .	33
Whole wheat, ground . . . . .	33
Ground gluten . . . . .	15
Gelatin . . . . .	15
Calcium carbonate ( $\text{CaCO}_3$ ) . . . . .	3
Sodium chloride ( $\text{NaCl}$ ) . . . . .	1

## 36.60

*Rachitogenic Diet No. 2*

	<i>per cent</i>
Whole yellow maize, ground . . . . .	76
Ground gluten . . . . .	20
Calcium carbonate ( $\text{CaCO}_3$ ) . . . . .	3
Sodium chloride ( $\text{NaCl}$ ) . . . . .	1

## VITAMIN D IN POULTRY FEED SUPPLEMENTS (8)—TENTATIVE

(Applicable to fish and fish liver oils and their extracts, and to materials used for supplementing vitamin D content of feeds. Not applicable to irradiated ergosterol products or to irradiated yeast unless recommended for poultry. This assay is comparison, under conditions specified, of efficacy of product under assay with that of U.S.P. Reference Cod Liver Oil in controlling ash content of bones of growing chicks.)

The basal ration is uniform mixture in proportions designated of following ingredients, which have been finely ground:

## 36.61

## BASAL RACHITIC RATION

	<i>per cent</i>
Ground yellow corn . . . . .	58
Wheat flour middlings or wheat gray shorts . . . . .	25
Crude domestic acid precipitated casein . . . . .	12
Calcium phosphate (precipitated) . . . . .	2
Iodized salt (0.02% KI) . . . . .	1
Non-irradiated yeast (7% minimum N) . . . . .	2
To each kg of above mixture add 0.2 g of $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ .	

## 36.62

## DETERMINATION

Provide cages with screen bottoms and keep chicks away from sunshine or other source of actinic light that may influence calcification. Keep cages in rooms in which wide variations in temp. are prevented (constant temp. preferred). Unless temp. of room is adequately controlled, provide each cage with suitable electrical heating device. Start all birds to be used in one assay on same day and keep all conditions of environment uniform for all groups in the assay.

Make assay on groups of one- or two-day-old white Leghorn chicks as specified below. Provide for one or more negative control groups that receive no vitamin D, three or more positive control groups that receive graduated levels of vitamin D from U.S.P. Reference Cod Liver Oil, and one or more assay groups for each product to be assayed. Have positive control and assay groups consist of not less than 20 birds each, and negative control group consist of not less than 10 birds. Make up rations for all groups in assay from one batch of basal ration. Add the Reference Cod Liver Oil to basal ration in such quantities as to produce measurable increase in percentage of bone ash above that obtained in negative control group (it is not possible to make comparisons if maximum bone ash is obtained). Add assay product to basal ration in such quantities as to permit direct comparison in response of assay and positive control groups. To basal ration of negative control group add corn oil equal in quantity to maximum quantity of oil fed to any group in assay, and add corn oil to rations of other groups until total quantity of corn oil and oil containing vitamin

D is equal to quantity of corn oil added to ration of negative control group. Feed chicks in respective groups prescribed ration and H<sub>2</sub>O (natural or distilled H<sub>2</sub>O) *ad libitum* for 21 days. Discard all chicks that show abnormality or disease not related to vitamin D deficiency. At least 15 chicks must remain in each reference or assay group that is used in calculating the vitamin D potency of an assay product.

Kill chicks; remove left tibia of each bird and clean of adhering tissue. (To facilitate removal of adhering tissue, bones may be placed in boiling H<sub>2</sub>O for not more than 2 min. Bones may be preserved in alcohol for extraction.) Completely extract bones with suitable fat solvent or solvents (20 hours with hot alcohol followed by 20 hours with ether is suitable, and bones may be crushed to facilitate extraction). Dry extracted bones to constant weight in moisture oven, cool in desiccator, and weigh. Ash moisture- and fat-free bones from each group of birds in muffle furnace to constant weight at any given temp. between 450 and 550°, or, if preferred, for 1 hour at ca 850°. (Ash determination may be made on individual bones if desired.) Cool ash in desiccator and weigh. Use consistently thruout any one assay the specific procedure adopted for extraction, drying, and ashing of the bones.

## 36.63

## INTERPRETATION OF RESULTS

One A.O.A.C. chick unit of vitamin D is equal in biological activity for the chick to one unit of vitamin D in the U.S.P. Reference Cod Liver Oil in this method of assay. The product under assay meets its declared vitamin potency in A.O.A.C. chick units of vitamin D if percentage of ash in moisture- and fat-free bone produced in assay groups by given number of units of vitamin D is equal to or greater than percentage of ash produced by same number of units of vitamin D from the U.S.P. Reference Cod Liver Oil.

## VITAMIN K

## 36.64

## BIOLOGICAL ASSAY (g)—TENTATIVE

(A comparison under specified conditions of the antihemorrhagic potency of any product with a chemically pure standard antihemorrhagic compound in controlling the blood prothrombin level of the blood of chicks.)

Place 1- or 2-day-old chicks that have not been fed in metal, wire-meshed-floored, electrically heated, battery brooders. Maintain temp. within battery compartments at 90–95°F. Have food and H<sub>2</sub>O receptacles outside the compartment, but available to chick thru apertures that permit only head of chick to pass; provide fresh H<sub>2</sub>O daily in clean troughs. To prevent bacterial synthesis discard any portion of ration that becomes wet, and use every precaution to prevent access of chicks to droppings. Discard any unused portion of ration every 4 days or oftener.

Provide all chicks with following basal ration, which must be finely ground and uniformly mixed:

	per cent
Sardine (pilchard) meal (1) .....	17.5
Dried brewer's yeast (1) .....	7.5
Ground polished rice .....	72.5
Cod-liver oil, U.S.P. ....	1.0
Calcium carbonate .....	0.5
Sodium chloride (2) .....	1.0

(1) Prepare by extracting continuously for 24 hours with ether.

(2) Add sufficient Mn in form of sulfate or carbonate to make 0.5% of the NaCl.

Maintain chicks on basal ration until clotting time of blood samples is 15 min. or more, as determined on 5% of chicks (usually 10–14 days). Following procedure may be used in determining clotting time:

Withdraw a few drops of blood by making small clean cut in exposed wing vein at junction of ulna, radius, and humerus. Put the blood in small vials and place in shaking device in thermostatically-controlled water bath, described later for determination of prothrombin clotting time. Measure time interval from withdrawal of blood to formation of firm clot.

When the chicks show the prescribed delayed clotting time divide them into uniform groups of not less than 12 each. Have at least 2 groups receiving different dosages of 2 methyl, 1,4 naphthoquinone as reference standard, and use at least one group for each product to be assayed. Maintain 1 group of 12 chicks on basal ration only to serve as negative control group.

Administer materials to be assayed and the standard orally as solns. Dissolve weighed or measured quantity of material to be administered in sufficient quantity of diluent so that desired daily dose is contained in 0.1 ml. If material to be administered is soluble in  $H_2O$ , use  $H_2O$  as diluent, and if it is fat-soluble use ethyl laurate. Administer a prescribed dose daily by mouth with tuberculin syringe or other equally suitable measuring device. In administering dose, open mouth of chick with pressure at corners of mouth, and permit soln to fall well down throat. Do not permit chicks to have access to food or  $H_2O$  for one-half hour after receiving the reference standard or assay products. Continue individual daily dose for 4 days at ca 24-hour intervals.

Approximately 24 hours after administration of last dose of standard and assay products determine prothrombin time on all chicks. Prepare clotting agent according to following procedure:

Select the 4 or 5 chicks to be used for this preparation at time assay is begun and maintain them on a practical chick ration containing at least 5% dried alfalfa. House chicks in manner to prevent contamination of rations of those receiving the standard or the assay product. Kill one of birds reserved for preparation of clotting agent by bleeding. Excise 10 g of breast muscle and grind with sand and 10 ml of 0.85% NaCl soln. Centrifuge mixture and filter thru very coarse paper. (Resulting liquid may be stored in refrigerator for several days, but preferably a fresh tissue extract should be used for each group of tests.) Dilute extract to 200 ml with 0.85% NaCl soln and then mix with equal volume of 0.025 *M*  $CaCl_2$  soln. (This mixture should clot blood of normal chicks reared on practical mash in 20–30 seconds when tested according to procedure described below. If it does not, concentration of clotting agent should be altered until prothrombin clotting time falls within this range. Blood of negative control chicks will usually fail to clot in less than 80 seconds.) Place 0.2 ml of 0.1 *M*  $Na_2C_2O_4$  soln in short and narrow tubes calibrated to 2 ml. Introduce 2 ml of blood from a chick into each tube. (Blood may be obtained conveniently by cutting off head of chick with scissors and directing the blood into tube with fingers.) Shake tube thoroly when blood has been drawn to the 2 ml mark. Pipet 0.1 ml samples of the oxalated blood into small cylindrical, flat-bottomed vials 15×50 mm. Add 0.2 ml of the clotting agent and simultaneously start a stop-watch. Place vial in thermostatically-controlled water bath adjusted to 38.5–39.0°. Use device to tilt vials at angle of ca 45° and back to vertical position once per second. When bottom of vial is covered by definite gelatinous film indicating clot formation, stop the watch. Lapse of time indicated by watch is prothrombin time. Duplicate procedure on blood from same chick until results do not vary by more than 2 seconds.

Calculate mean prothrombin time for each group. Plot reciprocal of mean prothrombin time against logarithm of dosage of vitamin K expressed in micrograms for groups receiving the 2 methyl, 1,4 naphthoquinone reference standard and connect points with straight line. Determine where reciprocal of mean prothrombin time for any group that received a product under assay intersects this line to determine



logarithm of vitamin K activity equivalent. 1 microgram of reference standard = 1 unit of vitamin K activity.

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## 37. WATERS, BRINE, AND SALT

### WATERS

#### POTABLE WATER

##### TURBIDITY (1)—OFFICIAL

###### 37.1

###### REAGENTS

(a) *Standard turbidity soln.*—Mix 1 g of elutriated fuller's earth, previously dried and sifted thru 200-mesh sieve, with  $H_2O$  and dilute to 1 liter. This stock mixture has turbidity of 1000. Check the stock soln with turbidimeter equipped with either candle or electric light.

(b) *Turbidity standards.*—Prepared by dilution of (a).

###### 37.2

###### DETERMINATION

If turbidity is 100 or more, use a turbidimeter; if less than 100 determine by direct comparison with turbidity standards, 37.1(b), contained in bottles of clear white glass.

##### COLOR—OFFICIAL

###### 37.3

###### REAGENTS

(a) *Standard color soln.*—Dissolve 1.245 g of  $K_2PtCl_6$  containing 0.5 g of Pt, and 1 g of  $CoCl_2 \cdot 6H_2O$  containing 0.25 g of Co, in 100 ml of  $H_2O$ ; add 100 ml of HCl and dilute to 1 liter with  $H_2O$ . This stock mixture has a color of 500.

(b) *Color standards.*—Prepared by dilution of (a).

###### 37.4

###### DETERMINATION

Compare color of sample, freed from suspended matter, with the standards in 50 ml Nessler tubes of clear white glass.

###### 37.5

###### ODOR—OFFICIAL

Shake vessel containing sample and note odor. Heat portion of sample to incipient boiling and note odor.

###### 37.6

###### TOTAL SOLIDS—OFFICIAL

Thoroughly shake vessel containing sample and pipet 100 ml of the unfiltered  $H_2O$  into weighed Pt dish. If sample contains much suspended matter, shake, pour rapidly into 100 ml measuring cylinder, and transfer without delay to weighed Pt dish. Evaporate to dryness and heat to constant weight at  $100^\circ$ .

###### 37.7

###### SOLIDS IN SOLUTION—OFFICIAL

Allow sample to stand until all sediment has settled and filter if necessary to secure a perfectly clear liquid. Occasionally a clear filtrate can be obtained only by use of alumina cream, but this should be avoided if possible. Evaporate 100–250 ml to dryness in weighed Pt dish. Heat to constant weight at  $100^\circ$ .

###### 37.8

###### IGNITED RESIDUE—OFFICIAL

Ignite residue from 37.6 until dish shows a dull red glow and ash is white or nearly so. Note any odor or change in color produced during ignition. Record weight of ignited residue and calculate loss on ignition.

## 37.9

## SUSPENDED MATTER—OFFICIAL

- (1) Subtract value for solids in soln, 37.7, from value for total solids, 37.6; or,  
(2) filter suitable measured volume of sample thru dry weighed Gooch crucible containing asbestos mat. Dry crucible and contents at 100°, cool, and weigh.

## NITROGEN IN FORM OF FREE AND ALBUMINOID AMMONIA

*Method I.—Official*

(For waters that do not contain hydrogen sulfide)

## 37.10

## REAGENTS

- ✓(a) *Nessler reagent*.—Dissolve 143 g of NaOH in 950 ml of H<sub>2</sub>O and filter thru asbestos. Add 50 g of red HgI<sub>2</sub> to filtrate and dilute with H<sub>2</sub>O to 1 liter. Mix thoroly, allow to settle, and use supernatant liquid.  
(b) *Alkaline potassium permanganate soln*.—Dissolve 143 g of NaOH and 8 g of KMnO<sub>4</sub> in H<sub>2</sub>O and dilute to 1 liter.  
(c) *Standard ammonium chloride soln*.—Dissolve 3.818 g of reagent-grade NH<sub>4</sub>Cl in NH<sub>3</sub>-free H<sub>2</sub>O and dilute to 1 liter. Then dilute 10 ml of this soln to 1 liter (1 ml = 0.01 mg of N, or 0.0128 mg of NH<sub>4</sub>.)

## 37.11

## DETERMINATION

Connect a flask of 1000 ml capacity with upright bulb condenser by means of half-inch glass tube and soft rubber stopper or recently extracted cork stopper. Place in flask 5 ml of saturated Na<sub>2</sub>CO<sub>3</sub> soln and 500 ml of NH<sub>3</sub>-free H<sub>2</sub>O. Distil and collect distillate into 50 ml Nessler tubes until no further traces of NH<sub>3</sub> are indicated on addition of 2 ml of the Nessler reagent to 50 ml of the distillate. Continue distillation until volume of soln in flask has been reduced to ca 200 ml. Cool slightly, add 500 ml of sample, and distil, at rate of ca 1 tubeful in 10 min., into 50 ml Nessler tubes until NH<sub>3</sub> ceases to be given off (4 or 5 tubes are usually sufficient). Add 2 ml of the Nessler reagent to each tube and let stand 10 min. From a small buret measure definite quantities of the NH<sub>4</sub>Cl soln into Nessler tubes. Dilute to 50 ml with NH<sub>3</sub>-free H<sub>2</sub>O, add 2 ml of the Nessler reagent, and compare depth of color with Nesslerized distillate. Report as mg/liter of N in the form of free NH<sub>3</sub>. Cool flask and add 50 ml of the alkaline KMnO<sub>4</sub> recently boiled. Distil and Nesslerize as directed above. Report as mg/liter of N in form of albuminoid NH<sub>3</sub>.

*Method II.—Official*

(For waters containing sulfur (2))

## 37.12

## REAGENTS

- (a) *Sulfuric acid soln*.—Dilute 7 ml of H<sub>2</sub>SO<sub>4</sub> (free from NH<sub>4</sub>-salts) to 500 ml.  
(b) *Sodium carbonate soln*.—Dissolve 66 g of anhydrous Na<sub>2</sub>CO<sub>3</sub> or 179 g of Na<sub>2</sub>CO<sub>3</sub>·10H<sub>2</sub>O in H<sub>2</sub>O and dilute to 250 ml.

The other reagents and solns used are described under 37.10.

## 37.13

## DETERMINATION

Place 500 ml of sample in a casserole, add 30 ml excess of the H<sub>2</sub>SO<sub>4</sub> soln, and boil carefully until free from sulfide (ca 20 min.). Place ca 300 ml of H<sub>2</sub>O and 8 ml of the Na<sub>2</sub>CO<sub>3</sub> soln in distilling flask connected as described under 37.11 and distil until

free from  $\text{NH}_3$ . Cool, add the cooled sample, which is now free from sulfide, and proceed with the distillation, addition of alkaline  $\text{KMnO}_4$  soln, etc., as directed under 37.11.

#### NITROGEN IN FORM OF NITRITE—OFFICIAL

##### 37.14

##### REAGENTS

(a) *Sulfanilic acid soln.*—Dissolve 1 g of sulfanilic acid in hot  $\text{H}_2\text{O}$ , cool, and dilute to 100 ml.

(b) *Alpha-naphthylamine hydrochloride soln.*—Boil 0.5 g of the salt with 100 ml of  $\text{H}_2\text{O}$ , kept at constant volume, for 10 min.

(c) *Standard nitrite soln.*—Dissolve 1.1 g of  $\text{AgNO}_2$  in  $\text{NO}_2$ -free  $\text{H}_2\text{O}$ , precipitate the Ag with NaCl soln, dilute to 1 liter, mix, and allow to settle. Dilute 100 ml to 1 liter and then 10 ml of this soln to 1 liter, using in each case  $\text{NO}_2$ -free  $\text{H}_2\text{O}$ . 1 ml of the last soln = 0.0001 mg of N (0.0003 mg of  $\text{NO}_2$ ).

##### 37.15

##### DETERMINATION

Place 100 ml of sample in 100 ml Nessler tube and add HCl dropwise until sample shows acid reaction to litmus paper. Add 1 ml of the sulfanilic acid soln and 1 ml of the alpha-naphthylamine hydrochloride soln, and thoroly mix. Set aside for 30 min. with other Nessler tubes containing known quantities of the  $\text{NO}_2$  soln made up to 100 ml with  $\text{NO}_2$ -free  $\text{H}_2\text{O}$  and acidified with HCl, the sulfanilic acid, and the alpha-naphthylamine hydrochloride solns, in the same manner as the sample. Determine quantity of N by comparing depth of pink color in the known and unknown solns. Record result as N.

#### NITROGEN IN FORM OF NITRATE (3)

##### 1. Phenoldisulfonic Acid Method—Official

(For water of low chlorine content)

##### 37.16

##### REAGENTS

(a) *Phenoldisulfonic acid soln.*—Dissolve 25 g of pure white phenol in 150 ml of  $\text{H}_2\text{SO}_4$ , add 75 ml of fuming  $\text{H}_2\text{SO}_4$  (13–15%  $\text{SO}_3$ ), and heat at  $100^\circ$  for 2 hours.

(b) *Standard nitrate soln.*—Dissolve 0.607 g of pure  $\text{NaNO}_3$  in 1 liter of  $\text{NO}_2$ -free  $\text{H}_2\text{O}$ . Evaporate 50 ml of this soln to dryness in porcelain dish; when cool, treat with 2 ml of the phenoldisulfonic acid soln, grind, and stir with glass rod to insure intimate contact; and dilute to 500 ml. 1 ml = 0.01 mg of N (0.04 mg of  $\text{NO}_3$ ). (This soln is permanent.) Prepare standards for comparison by adding  $\text{NH}_4\text{OH}$  to measured volumes of the standard soln in 100 ml Nessler tubes.

(c) *Standard silver sulfate soln.*—Dissolve 4.397 g of  $\text{Ag}_2\text{SO}_4$ , free from  $\text{NO}_3$ , in 1 liter of  $\text{H}_2\text{O}$ . 1 ml = 1 mg of Cl.

##### 37.17

##### DETERMINATION

To 100 ml of sample, or quantity that contains 0.05 mg or less of N, add sufficient standard  $\text{Ag}_2\text{SO}_4$  soln to precipitate all but ca 0.5 mg of the Cl. Heat to boiling and allow to settle, or add a little alumina cream, filter, and wash with small quantities of hot  $\text{H}_2\text{O}$ . Evaporate filtrate to dryness in porcelain dish on steam bath; when cool, treat with 2 ml of the phenoldisulfonic acid soln as directed under 37.16(b).

Dilute with  $H_2O$  and slowly add  $NH_4OH$  until maximum color is developed. Filter if necessary, transfer to colorimetric cylinder, and compare with the standards in usual manner. Record result as N.

## II. Reduction Method (4)—Official

(For water of high chlorine content)

### 37.18

#### REAGENTS

(a) *Aluminum foil*.—Should be purest obtainable. Cut into strips ca 10 cm long, weighing ca 0.5 g.

(b) *Sodium hydroxide soln*.—Dissolve 250 g of pure NaOH in 1250 ml of  $H_2O$ . Add 2 or 3 strips of the Al foil and let stand ca 12 hours. Concentrate the soln to 1 liter by boiling.

### 37.19

#### DETERMINATION

To 100 ml of sample, or quantity that contains 0.1 mg or less of N as  $NO_3$ , in 300 ml casserole, add 2 ml of the NaOH soln and concentrate by boiling to ca  $\frac{1}{2}$  original volume. Transfer to 100 ml test tube, using N-free  $H_2O$ , and dilute, if necessary, to ca 75 ml. Prepare a blank (preferably several blanks, since the N impurity in Al is often distributed unevenly) by placing ca 75 ml of N-free  $H_2O$  and 2 ml of the NaOH soln in 100 ml test tube. Place a strip of the Al foil in each tube. Close ends of test tubes with rubber stoppers connected by means of bent glass tubes with other test tubes containing ca 50 ml of slightly acidified  $NH_3$ -free  $H_2O$ . (These latter tubes serve as traps to prevent escape of  $NH_3$  and at the same time permit the free evolution of H.) Allow sample and blank to stand at room temp. for 12 hours or until reduction is complete. Nesslerize the traps. If high in  $NH_3$ , indicating frothing over of sample, discard determination. Disregard the traps if they contain only 0.01–0.02 mg of N as  $NH_3$  each. Transfer sample and blank to distillation flasks, using 250 ml of  $NH_3$ -free  $H_2O$  for each; distil, Nesslerize, and compare with standards as in determination of free  $NH_3$ , 37.11. Subtract quantity of N found in blank from that found in sample. Calculate to mg/liter of N

#### CHLORIDE—OFFICIAL

### 37.20

#### REAGENTS

(a) *Potassium chromate indicator*.—Dissolve 5 g of  $K_2CrO_4$  in  $H_2O$ , add saturated  $AgNO_3$  soln until slight permanent red precipitate is produced, filter, and dilute to 100 ml.

(b) *Standard silver nitrate soln*.—Dissolve 4.791 g of  $AgNO_3$  in  $H_2O$  and dilute to 1 liter. 1 ml = 1 mg of Cl. Check by titration against a standardized soln of NaCl.

### 37.21

#### DETERMINATION

To 100 ml of sample add a few drops of phenolphthalein indicator. If pink color appears, titrate the  $CO_2$  thus indicated to  $HCO_2$  with 0.05 N  $H_2SO_4$ . If sample is acid to methyl orange, add 0.05 N  $Na_2CO_3$  to neutralize acidity. Add 1 ml of the  $K_2CrO_4$  indicator and titrate with the standard  $AgNO_3$  soln. Correct for quantity of  $AgNO_3$  soln necessary to give, in 100 ml of Cl-free  $H_2O$  with 1 ml of the  $K_2CrO_4$  soln, the shade obtained at end of titration of sample. (If iodides and bromides are found in interfering quantities, make equivalent correction.)

If chlorides are present in very small quantities, concentrate 500 or 1000 ml in porcelain dish to 100 ml, rub down sides of dish carefully, add 1 ml of the  $K_2CrO_4$  indicator, and titrate with the standard  $AgNO_3$  soln. If sufficient chlorides are present in 100 ml of the water to consume more than 25 ml of the standard  $AgNO_3$  soln, determine by precipitation and weigh the  $AgCl$  as directed under 12.42.

## FLUORIDES (5)—OFFICIAL

## 37.22

## REAGENTS

(a) *Sodium fluoride soln.*—Dissolve 2.22 g of  $NaF$  (purity at least 98%) in 1 liter of  $H_2O$ . (This soln contains 1 mg of  $F/ml$ .)

(b) *Standard sodium fluoride soln.*—Dilute 10 ml of stock soln (a) to 1 liter. (1 ml = 0.01 mg of  $F$ .)

(c) *Thorium nitrate soln.*—Dissolve 0.25 g of  $Th(NO_3)_4 \cdot 12H_2O$  or 0.2 g of  $Th(NO_3)_4 \cdot 4H_2O$  in 1 liter of  $H_2O$ .

(d) *Alizarin red indicator.*—0.01%  $H_2O$  soln of  $Na$  alizarin sulfonate (alizarin red S).

(e) *Hydrochloric acid.*—Exactly 0.05  $N$ .

(f) *Sodium hydroxide soln.*—Exactly 0.05  $N$ .

(g) *Hydroxylamine hydrochloride soln.*—1.0%  $W/VNH_4OH.HCl$ .

## 37.23

## APPARATUS

(a) *Claisen flask.*—Capacity 250 ml.

(b) *Nessler tubes.*—6 long-form 50 ml tubes with double optically-plane disks fused to the tubes. Match tubes for length and test for optical similarity as follows: Add ca 40 ml of  $H_2O$ , 1 ml of the indicator, 2 ml of the  $HCl$ , and  $H_2O$  to mark on tube. Then to 1 tube add such a quantity of the  $Th(NO_3)_4$  soln that after making to mark and mixing, color is barely changed to faint pink. Note quantity of  $Th(NO_3)_4$  soln used. Add same quantity of  $Th(NO_3)_4$  to each of remaining 5 tubes. Reject tubes showing detectable differences in shade or intensity.

See also 29.24.

## 37.24

## PREPARATION OF SAMPLE

If sample has odor of  $H_2S$ , oxidize with 0.1 ml of 30%  $H_2O_2$  before evaporation.

Place 100 ml of sample in porcelain or  $Pt$  dish, make alkaline to phenolphthalein with 10%  $NaOH$  soln (avoid excess), and evaporate to 20 ml over Bunsen burner at temp. just below boiling point. During evaporation keep sample alkaline by adding small quantities of the  $NaOH$  soln from time to time. Transfer the 20 ml of evaporated sample to Claisen flask containing glass beads or boiling tube, which have been rinsed with boiling 10%  $NaOH$  soln to eliminate all traces of gelatinous  $SiO_2$  accumulating in flask.

Place flask containing sample on asbestos board ( $6'' \times 6'' \times \frac{1}{4}''$  with  $1''$  hole in center) over burner adjusted for medium sized flame. Close the straight neck of flask with 2-holed rubber stopper thru which pass a thermometer and the stem of a small separator, outlet of which is constricted to 2 mm diam. (Thermometer and outlet tube of separator should extend almost to bottom of flask.) Close other neck of flask with solid rubber stopper. Connect flask with water condenser, add 20 ml of 60%  $HClO_4$  to flask via the evaporating dish and separator, then add a quantity of saturated  $AgClO_4$  soln that will precipitate the chlorides (determined previously by titration with standard  $AgNO_3$  soln), and distil at  $132 \pm 3^\circ$ . Collect

nearly 200 ml of distillate. Make to volume (200 ml) and mix well. To determine acidity use 40 ml of distillate, add 1 ml of the indicator, mix thoroly, and note number of ml of the 0.05 *N* NaOH required for neutralization.

Repeat preparation and distillation, using 100 ml of H<sub>2</sub>O in place of sample, in order to ascertain the size of a blank.

## 37.25

## DETERMINATION

Prepare one standard, one color comparison tube, and one or more sample tubes as follows:

(a) *Color comparison tube*.—To 40 ml of H<sub>2</sub>O add 2 ml of the 0.05 *N* HCl, 1 ml of the alizarin red indicator, 1 ml of the NH<sub>2</sub>OH.HCl soln, and sufficient Th(NO<sub>3</sub>)<sub>4</sub> soln to give faint but definite pink end point. Compare all end point colors with this color.

(b) *Sample tube*.—To sample tube containing 40 ml of distillate add 1 ml of the indicator, 1 ml of the NH<sub>2</sub>OH.HCl soln, and such quantity of 0.05 *N* HCl that total amount of acid in tube (acidity previously determined plus quantity of 0.05 *N* HCl added) equals 2 ml of 0.05 *N* HCl. Make to volume and mix. If in the preliminary acidity determination it is found that the 40 ml distillate requires more than 2 ml of the NaOH soln for neutralization, do not add the HCl soln to the sample tube, but add to the standard tube same quantity of acid as was found present in sample tube. If 40 ml of the distillate requires more than 5 ml of 0.05 *N* NaOH, repeat distillation under conditions favorable to low acidity. From 10 ml buret, graduated to 0.05 ml, add the Th(NO<sub>3</sub>)<sub>4</sub> soln with frequent mixing until faint pink color appears, comparable to comparison tube (a). Note volume of Th(NO<sub>3</sub>)<sub>4</sub> soln used.

(c) *Standard tube*.—To standard tube containing 40 ml of H<sub>2</sub>O add 1 ml of the indicator, 1 ml of the NH<sub>2</sub>OH.HCl soln, and 2 ml or more of 0.05 *N* HCl, as was required in sample tube under (a). If aliquot chosen for determination already contains 2–5 ml of 0.05 *N* acid, add exactly the same amount to standard tube. Add exactly same quantity of the Th(NO<sub>3</sub>)<sub>4</sub> soln as was added to sample tube. To the standard tube (now more highly colored than sample tube) add the standard NaF soln from 10 ml buret with mixing until color matches that of sample tube. Make contents of both standard and sample tubes to same volume. Mix the soln in each tube and allow all air bubbles to escape before making color comparisons. Check end point by adding 1–2 drops of the NaF soln to the standard tube. Distinct color change should develop.

## 37.26

## CALCULATION

Subtract ml of NaF soln required by blank from ml of NaF soln required by sample.

$$\frac{\text{ml of NaF soln} \times \text{ml of total distillate} \times 10}{\text{ml aliquot titrated} \times \text{wt. of sample taken}} \cdot F \text{ (p.p.m.)}$$

**EXAMPLE:** A 100 ml sample, evaporated and distilled to 200 ml, of which a 40 ml aliquot corresponds to 5 ml of the NaF soln, gives:

$$\frac{5 \times 200 \times 10}{40 \times 100} = 2.5 F \text{ (p.p.m.)}$$

## OXYGEN REQUIRED

(By decomposition of organic matter present)

*Method I.—Official*

## 37.27

## REAGENTS

(a) *Standard potassium permanganate soln.*—Dissolve 0.395 g of  $\text{KMnO}_4$  in 1 liter of  $\text{H}_2\text{O}$ . Each ml has 0.1 mg of O available for oxidation.

(b) *Standard oxalic acid soln.*—Dissolve 0.788 g of  $\text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$  in 1 liter of  $\text{H}_2\text{O}$ .

Determine value of the oxalic acid soln in terms of the  $\text{KMnO}_4$  soln by boiling 10 ml of the oxalic acid and 200 ml of redistilled  $\text{H}_2\text{O}$  (prepared by treating distilled  $\text{H}_2\text{O}$  with alkaline  $\text{KMnO}_4$  and distilling) with 10 ml of  $\text{H}_2\text{SO}_4$  (1+3) and titrating, while still boiling, with the standard  $\text{KMnO}_4$  to appearance of pink color.

## 37.28

## DETERMINATION

Add 10 ml of  $\text{H}_2\text{SO}_4$  (1+3) to 200 ml of sample in porcelain dish and heat to boiling. Add from buret the standard  $\text{KMnO}_4$  until the water is distinctly red and boil for 10 min., adding more of the standard  $\text{KMnO}_4$  from time to time to maintain the red color. Add 10 ml of the standard oxalic acid and titrate back with the standard  $\text{KMnO}_4$  to pink color. From total number of ml of  $\text{KMnO}_4$  used, subtract number of ml equivalent to 10 ml of the oxalic acid. The result is number of ml of the  $\text{KMnO}_4$  required for 200 ml of the water. Correct for sulfides, nitrites, and ferrous salts, if present, by subtracting number of ml of the standard  $\text{KMnO}_4$  absorbed by another 200 ml portion of the sample when treated as above, except to digest at room temp. for 3 min.

*Method II(6)—Official*

(For water of high chloride content)

## 37.29

## REAGENTS

*Sodium hydroxide soln.*—Dissolve 50 g of  $\text{NaOH}$  in  $\text{H}_2\text{O}$ , cool, and make to 100 ml. The other reagents and solns used are described under 37.27.

## 37.30

## DETERMINATION

Introduce 100 ml of sample into 300 ml flask, add 0.5 ml of the  $\text{NaOH}$  soln and 10 ml of the  $\text{KMnO}_4$  soln, boil for 10 min., allow to cool to 50–60°, and add 5 ml of  $\text{H}_2\text{SO}_4$  (1+3) and 10 ml of the standard oxalic acid. As soon as the liquid has become perfectly colorless, and while constantly agitating, cautiously add the standard  $\text{KMnO}_4$  from buret dropwise, until liquid acquires faint permanent redness.  $\text{KMnO}_4$  used is quantity required for decomposition of organic matter in 100 ml of sample.

If 100 ml of the sample requires more than 4 ml of the  $\text{KMnO}_4$  for oxidation of organic matter, make second determination, using more of the  $\text{KMnO}_4$  and a correspondingly larger quantity of the  $\text{NaOH}$ , as undecomposed  $\text{KMnO}_4$  remaining after boiling must be at least twice as great as quantity decomposed.

## DISSOLVED OXYGEN (7)

*Method I.—Official*

(More than 0.1 mg of nitrite nitrogen/liter present)

## 37.31

## REAGENTS

(a) *Potassium permanganate soln.*—Dissolve 6.32 g of  $\text{KMnO}_4$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.



(b) *Potassium oxalate soln.*—Dissolve 20 g of  $K_2C_2O_4 \cdot H_2O$  in  $H_2O$  and dilute to 1 liter.

(c) *Manganous sulfate soln.*—Dissolve 480 g of  $MnSO_4 \cdot 4H_2O$  in  $H_2O$  and dilute to 1 liter.

(d) *Alkaline potassium iodide soln.*—Dissolve 500 g of NaOH and 150 g of KI in  $H_2O$  and dilute to 1 liter.

(e) *Sodium thiosulfate soln.*—0.025 *N*. Dissolve 6.205 g of A.C.S. reagent-grade  $Na_2S_2O_3 \cdot 5H_2O$  in  $H_2O$  and dilute to 1 liter with freshly boiled and cooled  $H_2O$ . 1 ml = 0.2 mg of O or 0.1400 ml of O at 0° and 760 mm pressure. As this soln is not permanent it should be standardized occasionally against 0.025 *N*  $K_2Cr_2O_7$  soln.

## 37.32

## COLLECTION OF SAMPLE

Collect sample in narrow-necked glass-stoppered bottle of 250–270 ml capacity by means of apparatus designed to avoid entrainment or absorption of any O from atmosphere. Note temp.

## 37.33

## DETERMINATION

Remove stopper from bottle, and add 0.7 ml of  $H_2SO_4$  and then 1 ml of the  $KMnO_4$  soln. Introduce these and all other reagents by pipet under surface of liquid. Insert stopper and mix by inverting bottle several times. If a noticeable excess of  $KMnO_4$  is not present after 20 min., again add 1 ml of the  $KMnO_4$  soln; if this is still insufficient, use a stronger  $KMnO_4$  soln. After 20 min. destroy excess of  $KMnO_4$  by adding 1 ml of the  $K_2C_2O_4$  soln, re-stopper bottle at once, and mix its contents. Add 1 ml of the  $MnSO_4$  soln and 3 ml of the alkaline KI soln. Allow precipitate to settle. Add 1 ml of  $H_2SO_4$  and mix by shaking. Transfer 200 ml of contents of bottle to flask and titrate with the 0.025 *N*  $Na_2S_2O_3$ , using a few ml of starch indicator, 6.3(e), toward the end of the titration. Do not add the starch soln until color has become faint yellow. Titrate until blue color disappears. Report results in mg/liter; if desired, report results also as percentage of saturation (8).

37.34 *Method. II.*—Official (less than 0.1 mg of nitrite nitrogen/liter present)

For reagents and collection of sample, see 37.31 and 37.32. Remove stopper from bottle and proceed as directed under 37.33, beginning "Add 1 ml of the  $MnSO_4$  soln."

## LEAD (8) (small quantities)

*Method I.*—Tentative (coloring matter, iron, copper, zinc present)

## 37.35

## REAGENTS

(a) *Ammonium acetate soln.*—Dissolve 200 g of  $NH_4$  acetate in  $H_2O$  and dilute to 500 ml. Soln should be practically colorless.

(b) *Dilute ammonium acetate soln.*—Dilute 50 ml of (a) to 500 ml.

(c) *Standard lead soln.*—Add  $H_2SO_4$  in slight excess to 10% Pb acetate soln. Filter off the  $PbSO_4$  and wash free from acid with  $H_2O$ . Dissolve the  $PbSO_4$  in the  $NH_4$  acetate soln, (a), dilute to definite volume, and determine the Pb as  $PbCrO_4$  by precipitating with  $K_2CrO_4$  soln, 6.41. Dilute stock soln so that 1 ml will contain 0.1 mg of Pb.

## 37.36

## REMOVAL OF COLOR

Acidify 0.5–2 liters of sample with HCl (1+1) and concentrate in porcelain casserole to volume of ca 75 ml by heating slowly over open flame. Add sufficient  $\text{NH}_4\text{Cl}$  (ca 2 g) to hold Mg in soln and assist in separation of sulfides. Add ca 1 ml of  $\text{NH}_4\text{OH}$  in excess and saturate with  $\text{H}_2\text{S}$ . Cover dish, allow to stand ca 2 hours, add more of the  $\text{NH}_4\text{OH}$  and  $\text{H}_2\text{S}$ , boil a few minutes, let precipitate settle, filter, and wash precipitate once with hot  $\text{H}_2\text{O}$ . (Precipitate will contain all the Fe, Pb, Cu, and Zn, and the coloring matter will be in filtrate.) Place filter and precipitate in small porcelain casserole, add 30 ml of  $\text{HNO}_3$  (1+3) and boil. Filter, wash free from acid, and cool filtrate (soln A).

## 37.37

## DETERMINATION

Add to soln A, 37.36, 5 ml of  $\text{H}_2\text{SO}_4$  (1+1), evaporate nearly to dryness, and heat cautiously until copious fumes of  $\text{SO}_3$  are given off. Cool, wash down sides of beaker with a little  $\text{H}_2\text{O}$ , and repeat evaporation and heating. Transfer to beaker with aid of  $\text{H}_2\text{O}$ , add equal volume of alcohol, and let stand overnight. Filter off the  $\text{PbSO}_4$  and wash with dilute alcohol, 50% by volume, until free from Fe. Collect filtrate, which contains Fe, Cu, and Zn, in 250 ml beaker (soln B). Digest filter containing the  $\text{PbSO}_4$  in small porcelain casserole with ca 40 ml of the warm  $\text{NH}_4$  acetate soln, filter, and wash once or twice with the warm dilute  $\text{NH}_4$  acetate soln and twice with  $\text{H}_2\text{O}$ . Dilute filtrate to definite volume. To aliquot add freshly prepared  $\text{H}_2\text{S}$  water and a few drops of acetic acid (1+1). Compare color obtained with a set of standards made by treating various quantities of the standard Pb soln with  $\text{H}_2\text{S}$  water.

**37.38** *Method II.—Tentative* (coloring matter present; 1 mg or less of iron present in quantity of sample taken for analysis; copper and zinc absent)

Remove coloring matter as directed under 37.36. Add to soln A 5 ml of  $\text{H}_2\text{SO}_4$  (1+1), evaporate nearly to dryness, and heat cautiously until copious fumes of  $\text{SO}_3$  are given off. Cool, wash down sides of container with a little  $\text{H}_2\text{O}$ , and repeat evaporation and heating. Transfer to beaker with aid of  $\text{H}_2\text{O}$ , add 25–40 ml of the  $\text{NH}_4$  acetate soln, 37.35(a), heat to boiling, and precipitate the Fe with  $\text{NH}_4\text{OH}$ . Filter and wash with the dilute  $\text{NH}_4$  acetate soln and  $\text{H}_2\text{O}$ . Acidify filtrate slightly with acetic acid (1+1) and determine Pb in filtrate colorimetrically by addition of freshly prepared  $\text{H}_2\text{S}$  water as directed under 37.37.

**37.39** *Method III.—Tentative* (coloring matter, iron, copper, zinc absent)

Add 5 ml of  $\text{H}_2\text{SO}_4$  (1+1) to 0.5–2 liters of sample, evaporate nearly to dryness, and heat until copious fumes of  $\text{SO}_3$  are given off. Transfer to beaker with aid of  $\text{H}_2\text{O}$ , add 25–40 ml of the  $\text{NH}_4$  acetate soln, 37.35(a), and determine Pb colorimetrically by addition of  $\text{H}_2\text{S}$  water as directed under 37.37.

**37.40** *Method IV.—Tentative* (coloring matter absent; iron, copper, zinc present)

Add 5 ml of  $\text{H}_2\text{SO}_4$  (1+1) to 0.5–2 liters of sample, evaporate nearly to dryness, and heat until copious fumes of  $\text{SO}_3$  are given off. Filter off the  $\text{PbSO}_4$  and proceed as directed under 37.37.

## COPPER—TENTATIVE (small quantities)

## 37.41

## REAGENTS

(a) *Ammonium nitrate soln.*—Dissolve 10 g of  $\text{NH}_4\text{NO}_3$  in  $\text{H}_2\text{O}$  and dilute to 100 ml.

(b) *Potassium ferrocyanide soln.*—Dissolve 3.5 g of  $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and dilute to 100 ml. This soln should be freshly prepared.

(c) *Standard copper soln.*—Dissolve ca 20 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ , add 1 ml of  $\text{H}_2\text{SO}_4$ , and dilute to 500 ml. Determine Cu in 50 ml of this soln as CuO by precipitation with KOH soln. Dilute stock soln so that 1 ml contains 0.1 mg of Cu.

## 37.42

## DETERMINATION

Boil the moderately acid filtrate (soln B), 37.37, which contains Fe, Cu, and Zn, to remove alcohol; adjust soln to 200 ml volume; and add 1 g of  $\text{NH}_4\text{Cl}$ . Heat to boiling, saturate with  $\text{H}_2\text{S}$ , and boil to remove precipitated S. Cover beaker, let stand ca 2 hours or until supernatant liquid becomes clear, filter, and wash the CuS without intermission with  $\text{H}_2\text{O}$  containing  $\text{H}_2\text{S}$ . Collect the filtrate, soln C, in porcelain casserole. Dissolve precipitate of CuS in hot  $\text{HNO}_3$  (1+3). Cool, add a few drops of phenolphthalein indicator, and make soln slightly alkaline with  $\text{NH}_4\text{OH}$  added carefully from dropping bottle. Add 10 ml of the  $\text{NH}_4\text{NO}_3$  soln, adjust volume to 100 ml, and boil gently until test with red litmus paper shows soln to be neutral. Filter soln to remove any Fe that may be present and adjust filtrate to volume of 100 ml. To an aliquot add 3 drops of the  $\text{K}_4\text{Fe}(\text{CN})_6$  soln. Compare color obtained with standards containing 0.1, 0.2, 0.3, 0.4, and 0.5 mg of Cu. Prepare these standards by measuring corresponding quantities of the standard Cu soln; adding phenolphthalein indicator, a slight excess of  $\text{NH}_4\text{OH}$ , and 10 ml of the  $\text{NH}_4\text{NO}_3$  soln; boiling the soln until neutral to red litmus, cooling, and adding 3 drops of the  $\text{K}_4\text{Fe}(\text{CN})_6$  soln. Make the colorimetric comparison in 100 ml Nessler jars.

## ZINC—TENTATIVE (small quantities)

## 37.43

## REAGENTS

(a) *Citric acid soln.*—Dissolve 50 g of citric acid crystals ( $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$ ) in  $\text{H}_2\text{O}$  and dilute to 100 ml.

(b) *Ammonium thiocyanate soln.*—Dissolve 20 g of  $\text{NH}_4\text{SCN}$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

(c) *Standard zinc soln.*—Dissolve pure Zn in HCl and dilute so that 1 ml contains 0.1 mg of Zn.

(d) *Potassium ferrocyanide soln.*—Dissolve 3.5 g of  $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and dilute to 100 ml.

## 37.44

## DETERMINATION

Boil the acid filtrate (soln C), 37.42, from the CuS precipitation to remove  $\text{H}_2\text{S}$ , cool, neutralize with  $\text{NH}_4\text{OH}$ , and add 10 ml of the citric acid soln. Heat to boiling, and if no Ca citrate separates add small quantities of powdered  $\text{CaCO}_3$  until precipitate of ca 1 g of Ca citrate is formed. Pass  $\text{H}_2\text{S}$  thru soln until it is cool. Let stand several hours, part of time on steam bath, until supernatant liquid is clear.

Filter, wash with the  $(\text{NH}_4)\text{SCN}$  soln and dissolve precipitate on filter with hot HCl (1+9). If filtrate is reddish in color, reprecipitate the Zn as before. Dispel turbidity of filtrate due to colloidal S by boiling. When filtrate is clear and colorless,

dilute an aliquot to 45 ml in 50 ml Nessler jar. Add 5 ml of the  $K_4Fe(CN)_6$  soln, mix quickly, and compare turbidity with standard Zn solns by viewing longitudinally the jars held over sheet of fine print. Prepare standards by mixing definite volumes of the standard Zn soln, 3 ml of HCl,  $H_2O$ , to make 45 ml, and 5 ml of the  $K_4Fe(CN)_6$  soln. The unknown soln should contain a volume of acid equivalent to that in standards. Do not use Zn borosilicate glassware in this determination.

### MINERAL WATER

37.45

#### SPECIFIC GRAVITY—OFFICIAL

Determine sp. gr. at 20/20° by means of pycnometer, 16.4.

37.46

#### SOLIDS IN SOLUTION—OFFICIAL.—See 37.7

37.47

#### IGNITED RESIDUE—OFFICIAL.—See 37.8

37.48

#### NITROGEN IN FORM OF FREE AND ALBUMINOID AMMONIA— OFFICIAL.—See 37.11 or 37.13

37.49

#### NITROGEN IN THE FORM OF NITRITE—OFFICIAL.—See 37.15

37.50

#### NITROGEN IN THE FORM OF NITRATE—OFFICIAL.—See 37.17 or 37.19

37.51

#### CHLORIDE—OFFICIAL.—See 37.21

37.52

#### FLUORIDES—OFFICIAL.—See 37.24–37.25

#### HYDROGEN SULFIDE (10)—OFFICIAL

37.53

#### REAGENTS

(a) *Iodine soln.*—0.02 *N.* Dissolve 10 g of KI (free from  $HIO_3$ ) in liter flask, using as little  $H_2O$  as possible. Add 2.54 g of resublimed I and dissolve by shaking. Dilute to mark with  $H_2O$ . Standardize against a  $Na_2S_2O_3$  soln that has been recently standardized against a  $K_2Cr_2O_7$  soln.

(b) *Iodine soln.*—0.01 *N.* Mix equal volumes of (a) and boiled  $H_2O$ . Standardize against a  $Na_2S_2O_3$  soln as directed under (a).

37.54

#### DETERMINATION

Transfer quantity of sample to graduated vessel by means of siphon and add a few drops of phenolphthalein indicator. If alkaline, add HCl until pink color of indicator disappears. Add starch indicator, 6.3(e), and with careful stirring titrate with the I soln, 37.53(a) or (b), until permanent blue color appears. Correct for quantity of I soln needed to give equally blue color. From corrected quantity of I soln used, calculate approximate quantity of  $H_2S$  present. For accurate determinations siphon 100–500 ml of sample, according to quantity of  $H_2S$  present, into graduated vessel, keeping outlet of siphon below liquid. Add immediately sufficient quantity of HCl, calculated from the approximate determination, to make neutral to phenolphthalein indicator. Mix carefully with bent glass rod, and without delay add ca 0.5 ml less I reagent (a) or (b) than is needed to combine with the  $H_2S$  present.

Add 5 ml of starch indicator, 6.3(e), and finish titration with the I soln dropwise with stirring until blue color remains permanently. Correct for quantity of I soln needed to give equally blue color when same quantity of starch soln is added to ca equal volume of boiled  $H_2O$ . If possible, make several determinations and take average. Standardize reagents (a) and (b) frequently.

## 37.55

## FREE CARBON DIOXIDE—TENTATIVE

If sample reacts acid to phenolphthalein and alkaline to methyl orange, titrate 100 ml with 0.05 *N* Na<sub>2</sub>CO<sub>3</sub> (free from bicarbonate) until soln is neutral to phenolphthalein. No. of ml used  $\times 1.1$  = mg of free CO<sub>2</sub> in 100 ml. Express results in mg/liter.

## 37.56

## CARBONIC AND BICARBONIC ACIDS—OFFICIAL

To 100 ml of sample add a few drops of phenolphthalein, and if pink color is produced titrate with 0.05 *N* HCl or H<sub>2</sub>SO<sub>4</sub>, adding a drop every 2–3 seconds until color disappears. Multiply buret reading by factor 3 to obtain mg of CO<sub>3</sub> ion in 100 ml. To the colorless soln from this titration, or to original soln if no color is produced with phenolphthalein, add 1 or 2 drops of methyl orange, continue titration without refilling buret, and note total reading. If CO<sub>3</sub> is absent, multiply total buret reading by factor 3.05 to obtain value of HCO<sub>3</sub> ion in mg/100 ml. If CO<sub>3</sub> is present, multiply reading with phenolphthalein by 2 and subtract from total reading of buret. Multiply difference by 3.05 to obtain HCO<sub>3</sub> ion in mg/100 ml. Express results as mg/liter.

## 37.57

## SILICA—OFFICIAL

Make preliminary examination, using 100–250 ml of sample, to determine approximate quantity of Ca and Mg present, in order to ascertain quantity of sample to be evaporated for final analysis.

Evaporate quantity sufficient to yield 0.1–0.6 g of CaO or 0.1–1 g of Mg<sub>2</sub>P<sub>2</sub>O<sub>7</sub> (usually 1–5 liters). Acidify the H<sub>2</sub>O with HCl and evaporate on steam bath to dryness in Pt dish. Continue drying ca 1 hour. Thoroughly moisten residue with 5–10 ml of HCl. Allow to stand 10–15 min. and add sufficient H<sub>2</sub>O to bring soluble salts into soln. Heat on steam bath until soln of salts is effected. Filter to remove most of the SiO<sub>2</sub> and wash thoroughly with hot H<sub>2</sub>O. Evaporate filtrate to dryness and treat residue with 5 ml of HCl and sufficient H<sub>2</sub>O to effect soln of soluble salts, as before. Heat, filter, and wash thoroughly with hot H<sub>2</sub>O. Designate filtrate as soln A. Transfer the 2 residues to Pt crucible, ignite, heat over blast lamp, and weigh. Moisten contents of crucible with a few drops of H<sub>2</sub>O. Add a few drops of H<sub>2</sub>SO<sub>4</sub> and a few ml of HF, and evaporate on steam bath under hood. Repeat treatment if all SiO<sub>2</sub> is not volatilized. Dry carefully on hot plate, ignite, heat over blast lamp, and weigh. Difference between 2 weights is weight of SiO<sub>2</sub>. Add weight of residue (Fe<sub>2</sub>O<sub>3</sub> + Al<sub>2</sub>O<sub>3</sub>) to that of Al<sub>2</sub>O<sub>3</sub> and Fe<sub>2</sub>O<sub>3</sub> obtained under 37.58. (If residue weighs more than 0.5 mg, BaSO<sub>4</sub> may be present in the water. If so, make necessary correction and add to weight of Fe<sub>2</sub>O<sub>3</sub> and Al<sub>2</sub>O<sub>3</sub> under 37.58.)

## 37.58

## IRON AND ALUMINUM—OFFICIAL

Concentrate soln A, 37.57, to 200 ml; while still hot, add NH<sub>4</sub>OH slowly, with constant stirring, until alkaline to methyl orange. Boil, filter, and wash 3 times with hot H<sub>2</sub>O. Dissolve precipitate in hot HCl (1+1). Dilute to ca 25 ml, boil, and again precipitate with NH<sub>4</sub>OH. Filter, wash thoroughly with hot H<sub>2</sub>O, dry, ignite, and weigh as Al<sub>2</sub>O<sub>3</sub> and Fe<sub>2</sub>O<sub>3</sub>. (In presence of H<sub>3</sub>PO<sub>4</sub>, weight of this residue must be corrected for the P<sub>2</sub>O<sub>5</sub> equivalent to the H<sub>3</sub>PO<sub>4</sub> found under 37.71, allowance being made for the difference in the volumes of the water used for these determinations.) Designate filtrate as soln B.

## IRON

**37.59** *Colorimetric Method—Official* (iron less than 1 mg; not applicable in presence of phosphates)

Fuse in Pt crucible the ignited precipitate of  $\text{Fe}_2\text{O}_3$  and  $\text{Al}_2\text{O}_3$ , 37.58, with fused  $\text{KHSO}_4$ , dissolve in  $\text{H}_2\text{O}$ , and precipitate the Fe and Al with  $\text{NH}_4\text{OH}$ . Dissolve precipitate on the filter paper in  $\text{HCl}$  and  $\text{HNO}_3$ , dilute soln, add 5%  $(\text{NH}_4)\text{SCN}$  soln, and compare color developed with that of calibrated color disks or standards containing known quantities of Fe.

**37.60**

*Volumetric Method—Official*

Fuse in Pt crucible the residue of  $\text{Fe}_2\text{O}_3$  and  $\text{Al}_2\text{O}_3$  with fused  $\text{KHSO}_4$ . This fusion takes but a few minutes, and must not be continued beyond time actually needed. When fusion is completed, set crucible aside and allow to cool. Add  $\text{H}_2\text{SO}_4$  (1+4) and heat crucible until fused mass is dissolved. Evaporate on steam bath as far as possible; then heat gradually until copious fumes of  $\text{SO}_3$  are given off. Dissolve in  $\text{H}_2\text{O}$  and allow to stand on steam bath. Cool, transfer to Erlenmeyer flask, and make up to such a volume that the soln does not contain more than 2.5% of free  $\text{H}_2\text{SO}_4$ . Pass  $\text{H}_2\text{S}$  thru the soln to reduce the Fe and precipitate any Pt contaminating residue from fusion. (Zn may be used instead of  $\text{H}_2\text{S}$  for reducing the Fe.) Filter, wash, and again pass  $\text{H}_2\text{S}$  thru soln so that all the Fe will be reduced. Expel the  $\text{H}_2\text{S}$  by boiling, at same time passing current of  $\text{CO}_2$  thru soln. Test escaping gas with Pb acetate paper to ascertain complete removal of  $\text{H}_2\text{S}$ . Discontinue boiling and let flask cool without discontinuing the current of  $\text{CO}_2$ . Titrate the reduced Fe with standard  $\text{KMnO}_4$  soln, 1 ml = 1 mg of Fe, and calculate as Fe.

**37.61**

## ALUMINUM—OFFICIAL

To obtain the weight of  $\text{Al}_2\text{O}_3$ , in absence of phosphates, subtract from the weight of  $\text{Fe}_2\text{O}_3$  and  $\text{Al}_2\text{O}_3$ , 37.58, the Fe, 37.59 or 37.60, calculated to  $\text{Fe}_2\text{O}_3$ . Calculate to Al.

**37.62**

## CALCIUM—OFFICIAL

Concentrate soln B, 37.58, to 150–200 ml, and to this soln, containing an equivalent of not more than 0.6 g of  $\text{CaO}$  or 1 g of  $\text{Mg}_2\text{P}_2\text{O}_7$ , add 1–2 g of oxalic acid and sufficient  $\text{HCl}$  (1+1) to clear the soln. Heat to boiling and neutralize with  $\text{NH}_4\text{OH}$ , stirring constantly. Add the  $\text{NH}_4\text{OH}$  in slight excess and allow to stand 3 hours in warm place. Filter off supernatant liquid and wash precipitate once or twice by decantation with 1%  $\text{NH}_4$  oxalate soln. Dissolve precipitate in  $\text{HCl}$  (1+1), dilute to 100–200 ml, add a little more oxalic acid, and precipitate as above. After allowing precipitate to stand 3 hours, filter, wash with the 1%  $\text{NH}_4$  oxalate soln, dry, ignite, heat over blast lamp, and weigh as  $\text{CaO}$  and  $\text{SrO}$ . Subtract from this weight, the weight of  $\text{SrO}$  equivalent to the Sr, 37.63. Difference is weight of  $\text{CaO}$ . Calculate to Ca. Designate combined filtrates and washings as soln C.

As a check on the  $\text{CaO}$ , evaporate to dryness the filtrate from the  $\text{Sr}(\text{NO}_3)_2$  under 37.63, beginning "Filter, and wash with ether-alcohol mixture, etc."; dissolve the  $\text{Ca}(\text{NO}_3)_2$  in  $\text{H}_2\text{O}$ , precipitate as oxalate, filter, wash, ignite, and weigh as  $\text{CaO}$ .  $\text{CaO} \times 0.7147 = \text{Ca}$ .

**37.63**

## STRONTIUM (11)—TENTATIVE

Dissolve the oxides, 37.62, in  $\text{HNO}_3$  (1+1) and test with spectroscope for Sr. If

Sr is present, transfer the  $\text{HNO}_3$  soln to small Erlenmeyer flask. Evaporate nearly to dryness over low flame and heat at  $150\text{--}160^\circ$  for 1–2 hours after the  $\text{H}_2\text{O}$  is evaporated. Break up the dried material with stirring rod and add 10–15 ml of a mixture of equal parts of absolute alcohol and ether to dissolve the  $\text{Ca}(\text{NO}_3)_2$ . Cork flask and allow to stand with frequent shaking for 2 hours or longer. Decant the soln thru 5.5 cm filter, reserving filtrate. Wash residue several times by decantation with small portions of ether-alcohol soln. Dry residue and filter paper and wash filter paper repeatedly with small portions of hot  $\text{H}_2\text{O}$ , collecting filtrate in the flask containing the main portion of the  $\text{Sr}(\text{NO}_3)_2$  residue. Add 1 or 2 drops of  $\text{HNO}_3$  (1+1), evaporate, dry, pulverize, and treat with 10–15 ml of the ether-alcohol mixture. Cork flask and let stand ca 12 hours with occasional shaking. Filter, and wash with ether-alcohol mixture until a few drops of filtrate evaporated on watch-glass leave practically no residue. Dry paper and precipitate. Dissolve the  $\text{Sr}(\text{NO}_3)_2$  in a few ml of hot  $\text{H}_2\text{O}$ . Add a few drops of  $\text{H}_2\text{SO}_4$  and then a volume of alcohol equal to volume of soln and allow to stand 12 hours. Filter, ignite, weigh as  $\text{SrSO}_4$ , and calculate to Sr. Test spectroscopically for Ca and Ba. If these elements are present, determine quantity and make necessary correction.

**37.64****MAGNESIUM—OFFICIAL**

Concentrate soln C, 37.62, to 200 ml, acidify with  $\text{HCl}$  (1+1), and add 2–3 g of  $(\text{NH}_4)_2\text{HPO}_4$  and sufficient  $\text{HCl}$  (1+1) to produce a clear soln when all the  $(\text{NH}_4)_2\text{HPO}_4$  is dissolved. When cold, make slightly alkaline with  $\text{NH}_4\text{OH}$ , stirring constantly. Add 2 ml excess of  $\text{NH}_4\text{OH}$  and allow to stand ca 12 hours. Filter off supernatant liquid and wash 4 times by decantation with  $\text{NH}_4\text{OH}$  (1+10). Dissolve precipitate in  $\text{HCl}$  (1+1), dilute to ca 150 ml, add a little  $(\text{NH}_4)_2\text{HPO}_4$ , and precipitate with  $\text{NH}_4\text{OH}$  as before. Allow to stand 12 hours, filter, wash free from chlorides with  $\text{NH}_4\text{OH}$  (1+10), place in porcelain crucible, ignite, heat over blast lamp, and weigh as  $\text{Mg}_2\text{P}_2\text{O}_7$ . Calculate to Mg.  $\text{Mg}_2\text{P}_2\text{O}_7 \times 0.21847 = \text{Mg}$ .

**37.65****SULFURIC ACID—OFFICIAL**

Make a preliminary examination, using 100–250 ml of sample, to determine approximate quantity of sulfates. (Alkali salts present can be approximated by calculating quantity of Na necessary to combine with the excess of acids— $\text{HCl}$ ,  $\text{H}_2\text{SO}_4$ , and  $\text{H}_2\text{CO}_3$ —over the Ca and Mg.)

Take a quantity (usually 1–5 liters) sufficient to yield not more than 1 g of  $\text{BaSO}_4$  and not more than 0.5 g of mixed chlorides. Acidify with  $\text{HCl}$  (1+1), evaporate to dryness in Pt dish, and remove  $\text{SiO}_2$  by two evaporations as directed under 37.57, using not more than 2 ml of  $\text{HCl}$  for the final soln. Combine filtrate and washings from the  $\text{SiO}_2$  determinations and concentrate to 150–200 ml. Heat to boiling and precipitate with slight excess of 10%  $\text{BaCl}_2$  soln, added very slowly and with constant stirring. Cover, and allow to stand on steam bath ca 12 hours. Filter, thoroly wash precipitate of  $\text{BaSO}_4$  with hot  $\text{H}_2\text{O}$  until free of chlorides, dry, ignite over Bunsen burner, and weigh.

If sulfate content of sample is unusually large, proceed as far as the concentration of the  $\text{SiO}_2$  filtrates, as directed above. Add 50 ml of  $\text{HCl}$ , heat to boiling, and precipitate with  $\text{BaCl}_2$  soln as before. Evaporate to dryness, take up in  $\text{H}_2\text{O}$  and a few drops of  $\text{HCl}$ , digest till precipitate settles, wash by decantation, filter, ignite, and weigh. Calculate to  $\text{SO}_4$  ion. Designate filtrate as soln E.

## SODIUM, POTASSIUM, AND LITHIUM

*Ether-Alcohol Method (12)—Official*

## 37.66

## PREPARATION OF MIXED CHLORIDES

Evaporate to dryness soln E, 37.65, in Pt dish, and ignite residue to faint redness to remove all traces of  $\text{NH}_4$  salts. Dissolve residue in dish in ca 200 ml of  $\text{H}_2\text{O}$  and precipitate with milk of lime or saturated  $\text{Ba}(\text{OH})_2$  soln. Boil, allow to stand for 30 min., and filter off the insoluble  $\text{Mg}(\text{OH})_2$  and undissolved  $\text{Ca}(\text{OH})_2$ . Thoroughly wash precipitate with hot  $\text{H}_2\text{O}$  and combine filtrate and washings. If the precipitate of Mg is large, dissolve it in a small quantity of HCl, evaporate to dryness, take up with  $\text{H}_2\text{O}$ , and precipitate as before. Concentrate the two filtrates and washings to 200–250 ml. Add  $\text{NH}_4\text{OH}$  and sufficient solid  $\text{NH}_4$  carbonate to precipitate the Ca and Ba. Allow to stand on steam bath for 1–2 hours. Filter off supernatant liquid, dissolve precipitate in HCl, reprecipitate as above, and wash thoroughly with hot  $\text{H}_2\text{O}$ . Evaporate combined filtrates and washings to dryness and drive off the  $\text{NH}_4$  salts by gentle heat. Treat residue with  $\text{H}_2\text{O}$ , pass thru small filter, using as little wash  $\text{H}_2\text{O}$  as possible, evaporate to small volume, and again precipitate with 1 or 2 drops of  $\text{NH}_4\text{OH}$  and 2 or 3 drops of saturated solns of  $\text{NH}_4$  carbonate and  $\text{NH}_4$  oxalate. If any precipitate appears, filter and repeat process. Evaporate filtrate to dryness and drive off all  $\text{NH}_4$  salts by heating to faint redness in Pt dish. Treat residue with a little  $\text{H}_2\text{O}$ , filter into small Pt dish, add a few drops of HCl (1+1), and evaporate to dryness. Dry in oven, then heat to faint redness, cool in desiccator, and weigh the combined chlorides of K, Na, and Li. Repeat heating to constant weight ( $x$ ). Dissolve mixed chlorides in hot  $\text{H}_2\text{O}$ , filter, and wash. Return filter paper and residue to dish, dry, ignite, and weigh ( $y$ ). Difference between ( $x$ ) and ( $y$ ) is weight of mixed chlorides.

## 37.67

## DETERMINATION

Dissolve the mixed chlorides, 37.66, in minimum quantity of cold  $\text{H}_2\text{O}$  (ca 1.5 ml will be more than sufficient for 0.5 g of the salts), introducing soln into tall 200 ml beaker. Add 1 drop of HCl, and then add gradually 20 ml of absolute alcohol, dropping the alcohol into center of beaker (not on sides) while rotating the soln. (The NaCl and KCl should be precipitated in perfectly uniform granular condition.) In similar manner, add 60 ml of ether (sp. gr. 0.716–0.717 at 25°) and allow mixture to stand ca 5 min. or until precipitate is well agglomerated and supernatant liquid almost clear, rotating mixture occasionally during this period. Filter thru weighed Gooch crucible into Erlenmeyer flask by means of suction, using bell jar arrangement, washing beaker thoroughly with mixture of 1 part alcohol and 5 parts ether, and collecting all precipitate on the Gooch with aid of policeman. After thoroughly washing precipitate on Gooch, set latter aside and rinse funnel with alcohol-ether mixture to wash any adhering Li soln into flask containing filtrate. Evaporate filtrate to dryness on steam bath, using air blast. Treat residue with 10 ml of absolute alcohol, warming if necessary, so that practically all residue dissolves. If a slight film remains on bottom and sides of flask, remove with policeman. Then, while rotating soln in flask, add 50 ml of ether (sp. gr. 0.716–0.717 at 25°), followed by 1 drop of HCl. Allow to stand for 30 min., rotating soln at frequent intervals. When the fine precipitate has agglomerated (only very small quantity is usually precipitated), filter into tall beaker by means of suction thru the Gooch crucible containing first precipitate. Wash combined precipitates with the ether-alcohol mixture, taking same precautions as in first precipitation. Dry Gooch and its contents in air, then in



oven, ignite gently, cool, and weigh to obtain combined weight of NaCl and KCl. Reserve crucible and contents for determination of K.

Evaporate on steam bath the ether-alcohol filtrate and washings containing the Li. Dissolve residue in a little  $H_2O$ , add slight excess of  $H_2SO_4$  (1+1), and transfer to weighed porcelain or Pt dish. Evaporate as far as possible on steam bath and then gently ignite residue over flame. (By placing dish on a triangle over asbestos gauze and using low flame, soln can be evaporated without spattering.) Finally ignite carefully over full flame, cool, and weigh. If charring has occurred, repeat ignition with  $H_2SO_4$ . Calculate to Li, using factor 0.1263.

Remove the KCl and NaCl from the Gooch crucible by washing with 25–50 ml of hot  $H_2O$ , using suction, and collecting filtrate in porcelain dish. Add sufficient Pt soln, 2.40(b), to convert the KCl and NaCl to  $K_2PtCl_6$  and  $Na_2PtCl_6$ , and evaporate to dryness. Treat residue with 80% alcohol by volume, filter, and wash until excess of  $PtCl_4$  and  $Na_2PtCl_6$  has been removed. Dry filter and precipitate, dissolve residue in hot  $H_2O$ , and transfer to weighed Pt dish. Evaporate on steam bath, dry for 30 min. in oven at  $100^\circ$ , cool, and weigh as  $K_2PtCl_6$ . Calculate to KCl, using factor 0.3067, and to K, using factor 0.1609.

Determine weight of NaCl by subtracting weight of KCl from weight of combined KCl and NaCl. Calculate to Na, using factor 0.3934.

#### BARIUM

(It is not necessary to look for Ba if sulfate is present in appreciable quantity unless the water contains a large quantity of bicarbonate or chloride, which may hold in soln a small quantity of both sulfate and Ba.)

#### Gravimetric Method (13)—Official

##### 37.68

##### REAGENTS

(a) *Ammonium dichromate soln.*—Dissolve 100 g of the salt free from  $SO_4$  in  $H_2O$  and dilute to 1 liter.

(b) *Ammonium acetate soln.*—Dissolve 300 g of the salt in  $H_2O$ , neutralize with  $NH_4OH$ , and dilute to 1 liter.

(c) *Dilute ammonium acetate soln.*—Dilute 20 ml of (b) to 1 liter.

Reaction of acetate solns should be alkaline rather than acid.

##### 37.69

##### DETERMINATION

Acidify a 1–5 liter portion of sample with HCl and concentrate to ca 200 ml. (If precipitate forms, filter it off and examine for Ba.) Add ca 0.5 g of  $NH_4Cl$  and precipitate the Fe and Al with  $NH_4OH$ . Boil, filter, and wash. To filtrate add an excess of the  $NH_4$  acetate soln (10 ml), keeping total volume ca 200 ml. Heat to boiling and add, with stirring, ca 5 ml of the  $(NH_4)_2Cr_2O_7$  soln. Allow to settle and cool. Decant clear liquid thru filter and wash precipitate by decantation with the dilute  $NH_4$  acetate soln until filtrate is no longer perceptibly colored (100 ml of wash soln). Place beaker under funnel, dissolve precipitate on paper with warm  $HNO_3$  (1+1), using as little as possible, and wash the paper. Add a little more acid to dissolve precipitate in beaker, then  $NH_4OH$  until precipitate that forms no longer redissolves. Heat to boiling; add, with stirring, 10 ml of the  $NH_4$  acetate soln and 2 ml of the  $(NH_4)_2Cr_2O_7$  soln; allow to cool slowly, and wash precipitate by decantation with the dilute  $NH_4$  acetate soln. Dry the  $BaCrO_4$ , burn filter separately, ignite moderately to constant weight, and weigh as  $BaCrO_4$ . Record as Ba, using factor 0.5422.

## 37.70

*Volumetric Method—Official*

Proceed as directed under 37.69 thru "wash precipitate by decantation with the dilute  $\text{NH}_4$  acetate soln" (after second precipitation). Then proceed as follows: Dissolve precipitate in ca 10 ml of  $\text{HCl}$  (1+1) and hot  $\text{H}_2\text{O}$ . Wash filter, dilute soln to ca 400 ml, and add ca 50 ml of freshly prepared 10%  $\text{KI}$  soln. Mix carefully and titrate the liberated  $\text{I}$  after 3 or 4 min. with 0.1  $N$   $\text{Na}_2\text{S}_2\text{O}_3$ . 1 ml of 0.1  $N$   $\text{Na}_2\text{S}_2\text{O}_3$  = 4.579 mg of  $\text{Ba}$ .

## 37.71

## PHOSPHORIC ACID—OFFICIAL

Treat 500 ml of sample, or larger quantity if necessary, with ca 10 ml of  $\text{HNO}_3$  and evaporate in porcelain dish nearly to dryness to drive off  $\text{HCl}$ . Treat residue with  $\text{H}_2\text{O}$  and filter if necessary. Add  $\text{NH}_4\text{OH}$  to alkalinity and then just enough  $\text{HNO}_3$  to restore acidity. Add some solid  $\text{NH}_4\text{NO}_3$  and heat in water bath at temp. of 45–50°. Add molybdate soln, 2.10(a), and keep at above temp. for 30 min. If more than trace of the yellow precipitate is present, filter and wash with recently boiled and cooled  $\text{H}_2\text{O}$  until entirely free from nitric and molybdic acids. Transfer precipitate and filter to beaker, add a little  $\text{H}_2\text{O}$ , and beat paper and contents to a pulp. Dissolve the yellow precipitate in small quantity of standard  $\text{KOH}$  soln, 2.10(b), add phenolphthalein indicator, 2.10(d), and titrate with the standard acid. From data so obtained calculate the  $\text{PO}_4$  ion to mg/liter.

## 37.72 PREPARATION OF SAMPLE—MANGANESE, IODINE, BROMINE, ARSENIC, AND BORIC ACID

Evaporate 0.5–2 liters of sample to dryness after addition of small quantities of solid  $\text{Na}_2\text{CO}_3$ . Boil residue thus obtained with  $\text{H}_2\text{O}$ , transfer to filter, and wash thoroly with hot  $\text{H}_2\text{O}$ . Use residue remaining on filter for determination of  $\text{Mn}$ . Dilute alkaline filtrate to definite volume and use for determination of  $\text{I}$ ,  $\text{Br}$ ,  $\text{As}$ , and  $\text{H}_3\text{BO}_3$ .

## MANGANESE

*I. Persulfate Method—Official*

## 37.73

## REAGENTS

(a) *Silver nitrate soln.*—Dissolve 2 g of  $\text{AgNO}_3$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

(b) *Standard manganous sulfate soln.*—Dissolve 0.2877 g of pure  $\text{KMnO}_4$  in ca 100 ml of  $\text{H}_2\text{O}$ , acidify soln with  $\text{H}_2\text{SO}_4$  (1+1), and slowly heat to boiling. Add slowly sufficient quantity of 10% oxalic acid soln to discharge the color. Cool, and dilute to 1 liter. 1 ml of this soln = 0.1 mg of  $\text{Mn}$ .

## 37.74

## DETERMINATION

Dissolve insoluble residue, 37.72, in excess of  $\text{HNO}_3$  (1+1), evaporate to dryness, treat with  $\text{H}_2\text{O}$ , and add ca 1 ml of  $\text{HNO}_3$  and a little of the  $\text{AgNO}_3$  soln. If precipitate of  $\text{AgCl}$  appears, add more of the  $\text{AgNO}_3$  until all the  $\text{Cl}$  is precipitated. Add excess of ca 10 ml of the  $\text{AgNO}_3$  soln for each mg of  $\text{Mn}$  present in sample. Filter, add 1 g of  $\text{NH}_4$  persulfate to filtrate, and place beaker or flask containing soln on steam bath until pink color develops (ca 20 min.). Compare color developed with standards similarly prepared by treating solns containing known quantities of the standard  $\text{MnSO}_4$  soln with the dilute  $\text{HNO}_3$ ,  $\text{AgNO}_3$  soln, and  $\text{NH}_4$  persulfate.

*II. Bismuthate Method (14)—Official***37.75****REAGENTS**

(a) *Nitric acid*.—(1+4). Free from brown oxide of N by aeration.

(b) *Dilute sulfuric acid*.—Dilute 25 ml of  $\text{H}_2\text{SO}_4$  to 1 liter with  $\text{H}_2\text{O}$ . Add enough  $\text{KMnO}_4$  soln to color faintly.

(c) *Standard manganous sulfate soln*.—Prepare as directed under 37.73(b). Soln of  $\text{KMnO}_4$  may be used in place of the  $\text{MnSO}_4$  soln. To prepare it dissolve 0.2877 g of  $\text{KMnO}_4$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

**37.76****DETERMINATION**

Remove Cl by several evaporations with  $\text{H}_2\text{SO}_4$  (1+1) from a quantity of the sample that contains 1 mg or less of Mn. Residue obtained under 37.72 may be used in place of fresh sample by dissolving it in excess of  $\text{HNO}_3$  (1+4), adding the dilute  $\text{H}_2\text{SO}_4$ , and removing Cl by two or more evaporations. In either case, volatilize the  $\text{H}_2\text{SO}_4$  and ignite residue at low heat (less than  $500^\circ$ ). Dissolve in 40 ml of  $\text{HNO}_3$  (1+3), add ca 0.5 g of the Na bismuthate, and heat until permanganate color disappears. Add a few drops of a 10% soln of  $\text{NH}_4$  bisulfite or saturated Na bisulfite to clear the soln and again boil to expel oxides of N. Remove from source of heat, cool to  $20^\circ$ , again add 0.5 g of the Na bismuthate, and stir. When maximum permanganate color has developed, filter thru alundum or Gooch crucible containing an asbestos mat that has been ignited, treated with a 4%  $\text{KMnO}_4$  soln and washed with  $\text{H}_2\text{O}$ . Wash precipitate with  $\text{H}_2\text{SO}_4$  (1+9) until washings are colorless. Transfer filtrate to colorimeter tube. Compare color developed with standards similarly prepared by treating solns containing known quantities of the standard  $\text{MnSO}_4$  with the dilute  $\text{HNO}_3$ ,  $\text{NH}_4$  or Na bisulfite soln, and Na bismuthate. Color may also be compared with that of standards prepared from the  $\text{KMnO}_4$  soln by diluting portions of 0.2, 0.4, 0.6 ml, etc., of the  $\text{KMnO}_4$  soln with the dilute  $\text{H}_2\text{SO}_4$  to same volume as filtrate.

**IODIDE AND BROMIDE—TENTATIVE**

(This method is qualitative and approximately quantitative. For accurate quantitative methods for iodides, see 37.118.)

**37.77****REAGENTS**

(a) *Sodium hydroxide soln*.—Dissolve 10 g of NaOH in  $\text{H}_2\text{O}$ , cool, and dilute to 100 ml.

(b) *Sodium nitrite soln*.—Dissolve 2 g of  $\text{NaNO}_2$  in  $\text{H}_2\text{O}$  and dilute to 1 liter.

**37.78****DETERMINATION**

Evaporate to dryness an aliquot of alkaline filtrate, 37.72; add 2–3 ml of  $\text{H}_2\text{O}$  to dissolve residue and enough alcohol to make percentage of alcohol ca 90. (This precipitates the chlorides.) Heat to boiling, filter, and repeat preceding soln and precipitation once or twice. Add 2 or 3 drops of the NaOH soln to combined alcoholic filtrates and evaporate to dryness. Dissolve this last residue in 2–3 ml of  $\text{H}_2\text{O}$  and repeat precipitation with alcohol, heating, and filtering. Add a drop of the NaOH soln to this alcoholic filtrate and evaporate to dryness. Dissolve this residue in a little  $\text{H}_2\text{O}$ ; acidify with  $\text{H}_2\text{SO}_4$  (1+5), using 3 or 4 drops in excess; and transfer to small flask. Add 4 drops of the  $\text{NaNO}_2$  soln and ca 5 ml of  $\text{CS}_2$ . Shake until all the I is

extracted and filter off the acid soln from the  $\text{CS}_2$ . Wash flask, filter and contents with cold  $\text{H}_2\text{O}$  and transfer the  $\text{CS}_2$  containing the I in soln to Nessler tube, using ca 5 ml of  $\text{CS}_2$ . In washing filter, make contents of tube up to definite volume, usually 12–15 ml, and compare color with that of other tubes containing known quantities of I dissolved in  $\text{CS}_2$ . Prepare these standard tubes by treating measured quantities of a soln of known KI content as described above, beginning "acidify with  $\text{H}_2\text{SO}_4$  (1+5)."

Transfer separately to small flasks the acid soln of the sample and the standards from which the I has been removed. To standards add definite measured quantities of a bromide soln of known strength, and to each of flasks containing sample and standards add 5 ml of  $\text{CS}_2$ . Add saturated and freshly prepared Cl water, 1 ml at a time, shaking after each addition until all the Br is set free. Avoid a large excess of the Cl, as a bromo-chloride may form and spoil the color reaction. Filter off the  $\text{H}_2\text{O}$  soln from the  $\text{CS}_2$  thru moistened filter, wash contents of filter 2 or 3 times with  $\text{H}_2\text{O}$ , and then transfer to Nessler tube by means of ca 1 ml of  $\text{CS}_2$ . Repeat this extraction of filtrate twice, using 3 ml of  $\text{CS}_2$  each time. Combined  $\text{CS}_2$  extracts usually amount to 11.5–12 ml. Add enough  $\text{CS}_2$  to tubes to bring them to definite volume, usually 12–15 ml, and compare sample with standards. If, when using this method near its upper limit, quantities of  $\text{CS}_2$  recommended do not extract all the Br, make one or two extra extractions with  $\text{CS}_2$ ; transfer extracts to another tube; and compare color with some of lower standards. Add readings thus obtained to the others.

Results closely approximating true values for I and Br can be obtained in a shorter time on most samples by omitting the extractions with alcohol and comparing the color of the  $\text{CS}_2$  solns directly in the extraction flasks.

#### ARSENIC—OFFICIAL

37.79

REAGENTS AND APPARATUS.—See 29.1 and 29.2

37.80

#### DETERMINATION

Take a portion of alkaline filtrate, 37.72, that contains not more than 0.03 mg of  $\text{As}_2\text{O}_3$ . If quantity taken is greater than 10 ml, evaporate soln to ca that volume on steam bath. Transfer soln into the generator of the apparatus described under 29.2, with aid of ca 10 ml of  $\text{H}_2\text{O}$ , add 20 ml of  $\text{H}_2\text{SO}_4$  (1+2), and proceed as directed under 29.5, beginning "add 5 ml of the KI reagent."

37.81

#### BORIC ACID—OFFICIAL

(Glassware containing boron must not be used in this determination)

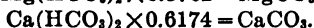
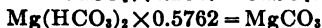
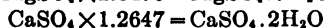
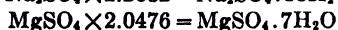
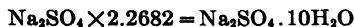
*Qualitative test.*—Evaporate to dryness a part of the alkaline filtrate, 37.72, treat with 1–2 ml of  $\text{H}_2\text{O}$ , and slightly acidify with HCl (1+1). Add ca 25 ml of alcohol, boil, filter, and repeat extraction of residue. Make filtrate slightly alkaline with NaOH soln and evaporate to dryness. Add a little  $\text{H}_2\text{O}$ , slightly acidify with the dilute HCl, and place strip of turmeric paper in liquid. Evaporate to dryness on steam bath and continue heating until turmeric paper is dry. If  $\text{H}_3\text{BO}_3$  is present, turmeric paper takes on cherry-red color. As confirmatory test, apply a drop of  $\text{NH}_4\text{OH}$  (1+1) to the reddened paper. Dark olive color will be due to boric acid, 32.17.

*Quantitative method.*—Use the Gooch method (15).

## 37.82

METHOD OF REPORTING RESULTS IN WATERS AND  
BRINE (16)—OFFICIAL

Report radicals and anhydrous salts in terms of mg/liter or, in case of highly concentrated waters, in terms of g/liter. For the benefit of physicians, in the case of medicinal waters, report also the salts in terms of grains/quart, using factor 0.014600 to convert mg/liter to grains/quart. In reporting salts in terms of grains/quart, convert the salts that have H<sub>2</sub>O of crystallization to the hydrated form as expressed in U. S. Pharmacopoeia and in National Formulary, and convert the Mg(HCO<sub>3</sub>)<sub>2</sub> to MgCO<sub>3</sub> and Ca(HCO<sub>3</sub>)<sub>2</sub> to CaCO<sub>3</sub>. Use following factors in these calculations:



When a complete analysis is made report error of analysis and state how it is distributed. Report only significant figures.

Report Fe and Al together when present in unimportant quantities and in calculations consider them as Fe. When Fe and Al are present in larger quantities, make the separation and report each separately.

In calculating the hypothetical combinations of acid and basic ions, join NO<sub>2</sub>, NO<sub>3</sub>, BO<sub>2</sub>, and AsO<sub>4</sub> to Na; I and Br to K; and PO<sub>4</sub> to Ca. Assign the residual basic ions in the following order: NH<sub>4</sub>, Li, K, Na, Mg, Ca, Sr, Mn, Fe, and Al, to the residual acid ions in the following order: Cl, SO<sub>4</sub>, CO<sub>3</sub>, and HCO<sub>3</sub>. In case HCO<sub>3</sub> is not present in sufficient quantity to join with all the Ca, the residual Ca is joined to SiO<sub>2</sub> to form CaSiO<sub>3</sub>, and Mn, Fe, and Al are calculated to the oxides Mn<sub>2</sub>O<sub>4</sub>, Fe<sub>2</sub>O<sub>3</sub>, and Al<sub>2</sub>O<sub>3</sub>, respectively.

Use equivalent combining weights or their reciprocals in uniting the radicals, and when necessary for the purpose of comparison, in reducing salts to radicals and reuniting the radicals in the order specified above.

The equivalent combining weight of a radical is obtained by dividing its weight by its valence. The equivalent combining weight of a salt is obtained by dividing its molecular weight by the product of the valency of the basic element and the number of atoms of the basic element in the salt.

The procedure in calculating the hypothetical combinations by the use of the equivalent combining weights and their reciprocals is as follows:

Multiply the weights obtained, expressed in mg/liter, or, in the case of highly concentrated waters, in g/liter, for each radical to be combined, by the corresponding reciprocal of the equivalent combining weights. If the Na and K are to be determined by calculation, as is frequently the case, subtract the sum of the values obtained (reacting values) for the basic radicals from the sum of the reacting values for the acid radicals. The difference represents the reacting value of the undetermined Na and K. When all the constituents in the water have been determined the sums of the reacting values of the acid and of the basic radicals should be very nearly the same. In this case, if the difference is reasonable and well within the limit of accuracy of the methods used, it may be distributed equally among all the radicals determined, or among those that the analyst believes to be less accurate than the others. If the difference is unreasonably great, repeat the analysis in whole or in part. The sums of the reacting values of the acid and basic radicals must be equal before proceeding with the calculation. Obtain the reacting values of the salts by

subtracting in succession the reacting values of the radicals in the specified order. To convert these values to mg/liter of the respective salts multiply each of them by the equivalent combining weight of the respective salt.

**37.83**      *Equivalent Combining Weights and Their Reciprocals Based on International Atomic Weights, 1948*

NEGATIVE RADICALS	EQUIVALENT COMBINING WEIGHTS	RECIPROCAL OF EQUIVALENT COMBINING WEIGHTS	POSITIVE RADICALS	EQUIVALENT COMBINING WEIGHTS	RECIPROCAL OF EQUIVALENT COMBINING WEIGHTS
NO <sub>3</sub>	62.008	0.01613	NH <sub>4</sub>	18.0404	0.05543
BO <sub>2</sub>	42.82	0.02335	Li	6.940	0.14409
AsO <sub>4</sub>	46.30	0.02160	K	39.096	0.02558
I	126.92	0.00788	Na	22.997	0.04348
Br	79.916	0.01251	Mg	12.16	0.08224
PO <sub>4</sub>	31.660	0.03158	Ca	20.04	0.04990
HS	33.0681	0.03024	Sr	43.815	0.02282
S	16.03	0.06238	Ba	68.68	0.01456
SiO <sub>3</sub>	38.03	0.02630	Mn	27.465	0.03641
O	8.0000	0.12500	Fe <sup>II</sup>	27.92	0.03582
Cl	35.457	0.02820	Fe <sup>III</sup>	18.613	0.05372
SO <sub>4</sub>	48.03	0.02082	Al	8.99	0.11123
CO <sub>3</sub>	30.005	0.03333	Cu	31.785	0.03146
HCO <sub>3</sub>	61.018	0.01639			

SALTS	EQUIVALENT COMBINING WEIGHTS	RECIPROCAL OF EQUIVALENT COMBINING WEIGHTS	SALTS	EQUIVALENT COMBINING WEIGHTS	RECIPROCAL OF EQUIVALENT COMBINING WEIGHTS
NH <sub>4</sub> Cl	53.4974	0.01869	MgSO <sub>4</sub>	60.19	0.01661
LiCl	42.397	0.02359	MgCO <sub>3</sub>	42.16	0.02372
Li <sub>2</sub> SO <sub>4</sub>	54.970	0.01819	Mg(HCO <sub>3</sub> ) <sub>2</sub>	73.158	0.01366
Li <sub>2</sub> CO <sub>3</sub>	36.945	0.02707	Mg(NO <sub>3</sub> ) <sub>2</sub>	74.168	0.01348
LiHCO <sub>3</sub>	67.958	0.01472	CaCl <sub>2</sub>	55.497	0.01802
KCl	74.553	0.01341	CaSO <sub>4</sub>	68.07	0.01469
K <sub>2</sub> SO <sub>4</sub>	87.126	0.01148	CaCO <sub>3</sub>	50.045	0.01998
K <sub>2</sub> CO <sub>3</sub>	69.101	0.01447	Ca(HCO <sub>3</sub> ) <sub>2</sub>	81.058	0.01234
KHCO <sub>3</sub>	100.114	0.00999	CaSiO <sub>3</sub>	58.07	0.01722
KI	166.016	0.00602	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	51.70	0.01934
KBr	119.012	0.00840	SrSO <sub>4</sub>	91.845	0.01089
NaCl	58.454	0.01711	SrCO <sub>3</sub>	73.82	0.01355
NaBr	102.913	0.00972	Sr(HCO <sub>3</sub> ) <sub>2</sub>	104.833	0.00954
NaI	149.917	0.00667	BaSO <sub>4</sub>	116.71	0.00857
Na <sub>2</sub> SO <sub>4</sub>	71.027	0.01408	Ba(HCO <sub>3</sub> ) <sub>2</sub>	129.698	0.00771
Na <sub>2</sub> CO <sub>3</sub>	53.002	0.01887	MnSO <sub>4</sub>	75.495	0.01324
NaHCO <sub>3</sub>	84.0151	0.01190	MnCO <sub>3</sub>	57.47	0.01740
NaNO <sub>3</sub>	69.005	0.01449	Mn(HCO <sub>3</sub> ) <sub>2</sub>	88.483	0.01130
NaNO <sub>2</sub>	85.005	0.01176	FeSO <sub>4</sub>	75.95	0.01317
NaBO <sub>3</sub>	65.817	0.01519	Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	66.643	0.01500
Na <sub>2</sub> AsO <sub>4</sub>	69.300	0.01443	FeCO <sub>3</sub>	57.925	0.01726
NaF	41.997	0.02381	Fe(HCO <sub>3</sub> ) <sub>2</sub>	88.943	0.01124
NaHS	56.0651	0.01784	Fe <sub>2</sub> O <sub>3</sub>	26.613	0.03758
Na <sub>2</sub> PO <sub>4</sub>	54.657	0.01829	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	57.02	0.01754
Na <sub>2</sub> S	39.027	0.02562	Al <sub>2</sub> O <sub>3</sub>	16.99	0.05886
Na <sub>2</sub> SiO <sub>3</sub>	61.027	0.01639			
MgCl <sub>2</sub>	47.617	0.02100			

**INDUSTRIAL WATER**

## 37.85

CHLORIDE—OFFICIAL.—*See* 37.21

## 37.86

FLUORIDES—OFFICIAL.—*See* 37.22–37.26  
and if organic material is present *see also* 29.24–29.28.

## 37.87

CARBONIC AND BICARBONIC ACIDS—OFFICIAL.—*See* 37.56

## 37.88

NITRATES—OFFICIAL.—*See* 37.17 or 37.19

## 37.89

## SILICA—OFFICIAL

Proceed as directed under 37.57. Generally one evaporation with HCl for removal of  $\text{SiO}_2$  is sufficient.

## 37.90

IRON AND ALUMINUM—OFFICIAL.—*See* 37.58

## 37.91

## CALCIUM—OFFICIAL

If no  $\text{H}_3\text{PO}_4$  is present, concentrate the filtrate from the determination of Fe and precipitate with  $\text{NH}_4\text{OH}$  and oxalic acid as directed under 37.62. (Usually one precipitation is sufficient.)

## 37.92

MAGNESIUM—OFFICIAL.—*See* 37.64

## 37.93

## SULFURIC ACID AND ALKALIES—OFFICIAL

Proceed as directed under 37.65–37.67. For technical purposes sufficient accuracy is obtained by determining the acids and the bases, except Na and K, and then calculating the excess of acid over basic ions to the Na salt, stating the alkali thus found as Na and K by difference.

## 37.94

## TEMPORARY HARDNESS—OFFICIAL

The difference between the alkalinity after boiling, 37.95(b), and the alkalinity before boiling, 37.95(a), is the temporary hardness in p.p.m. of  $\text{CaCO}_3$ .

## 37.95

## ALKALINITY (17)—OFFICIAL

(a) *Before Boiling—Official.*—Measure 100 ml of sample into 250 ml clear glass-stoppered bottle; add 2.5 ml of erythrosine indicator (0.1 g of the Na salt in 1 liter of  $\text{H}_2\text{O}$ ), 5 ml of  $\text{CHCl}_3$  (neutral to erythrosine), and 0.02 N  $\text{H}_2\text{SO}_4$  in small quantities, shaking bottle vigorously after each addition of acid. Rose color gradually disappears and is finally discharged by 1 or 2 drops of the acid. White paper held back of the bottle facilitates detection of end point. Multiply number of ml of 0.02 N  $\text{H}_2\text{SO}_4$  used by 10 to obtain number of p.p.m. of alkalinity in terms of  $\text{CaCO}_3$ .

(b) *After Boiling—Official.*—Boil 100 ml of sample in porcelain dish gently for 30 min. Cool, transfer to 100 ml volumetric flask, and fill to mark with recently boiled and cooled  $\text{H}_2\text{O}$ . Filter thru dry paper and determine alkalinity of filtrate as directed under (a), making proper calculation for aliquot used and calculating in terms of  $\text{CaCO}_3$  the p.p.m. of alkalinity after boiling.

## 37.96

## TOTAL HARDNESS (18)—OFFICIAL

Add sufficient 0.05 N  $\text{H}_2\text{SO}_4$  to 200 ml of sample contained in 500 ml Pyrex or similar glass Erlenmeyer flask to neutralize the alkalinity, quantity required being calculated from results obtained as directed under 37.95(a). Measure 200 ml of  $\text{H}_2\text{O}$  into similar flask. Treat contents of each flask in following manner: Boil 15

min. to expel free  $\text{CO}_2$ , add 25 ml of soda reagent (0.1 *N*, equal parts of  $\text{NaOH}$  and  $\text{Na}_2\text{CO}_3$ ), boil 10 min., cool, rinse into 200 ml volumetric flask, and dilute to 200 ml with boiled  $\text{H}_2\text{O}$ . Filter, rejecting first 50 ml, and titrate 50 ml of each filtrate with 0.05 *N*  $\text{H}_2\text{SO}_4$  in presence of methyl orange or erythrosine indicator. Total hardness (p.p.m. of  $\text{CaCO}_3$ ) = 50 times difference between ml of 0.05 *N*  $\text{H}_2\text{SO}_4$  used in titrating aliquot of blank and aliquot of sample.

### 37.97 PERMANENT OR NON-CARBONATE HARDNESS—OFFICIAL

Difference between alkalinity before boiling, 37.95(a), and total hardness, 37.96, = permanent or non-carbonate hardness (p.p.m. of  $\text{CaCO}_3$ ).

## IRRIGATING WATER

### 37.98 GENERAL METHODS—OFFICIAL

Determine the solids in soln,  $\text{Cl}$ ,  $\text{F}$ ,  $\text{CO}_3$  and  $\text{HCO}_3$ ,  $\text{Ca}$ ,  $\text{Mg}$ , and  $\text{H}_2\text{SO}_4$  as directed under 37.7, 37.21, 37.22, 37.56, 37.62, 37.64, and 37.65, respectively. To make the hypothetical combination, calculate  $\text{Ca}$  and  $\text{Mg}$  to the acid ions in following order:  $\text{HCO}_3$ ,  $\text{SO}_4$ , and  $\text{Cl}$ . Then calculate remaining acid ions, including  $\text{CO}_3$ , to corresponding salts of  $\text{Na}$ .

### BLACK ALKALI (19)—OFFICIAL

#### 37.99

#### REAGENTS

(a) *Sodium carbonate*.—0.02 *N*. 1 ml = 0.00106 g of  $\text{Na}_2\text{CO}_3$ .

(b) *Carbon dioxide-free water*.—Boil  $\text{H}_2\text{O}$  vigorously until ca  $\frac{1}{3}$  of original volume is evaporated, cool, and stopper.

#### 37.100

#### DETERMINATION

Transfer 200 ml of sample to Pt or Ag dish; add 50–100 ml of the  $\text{Na}_2\text{CO}_3$  soln, according to quantity of soluble salts of  $\text{Ca}$  and  $\text{Mg}$  present; and evaporate to dryness. Rub up residue with  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  and transfer to 100 ml volumetric flask. (Ordinary laboratory wash bottle should not be used to transfer residue, as  $\text{CO}_2$  from breath of operator will vitiate results.) Dilute to mark, shake thoroly, and allow to stand until clear (12–15 hours). Remove 50 ml of the clear supernatant liquid, equivalent to half original quantity of sample and  $\text{Na}_2\text{CO}_3$  added, and transfer to 250 ml glass-stoppered flask or stoppered titration bottle of clear glass without any tinge of pink. Add 5 ml of  $\text{CHCl}_3$ , neutral to erythrosine, and 1 ml of erythrosine indicator (0.1 g of the  $\text{Na}$  salt in 1 liter of  $\text{H}_2\text{O}$ ) and titrate with 0.02 *N*  $\text{H}_2\text{SO}_4$  until color disappears. Shake soln vigorously after each addition of acid. Milky appearance produced by the  $\text{CHCl}_3$  makes reading of end point sharp and certain.

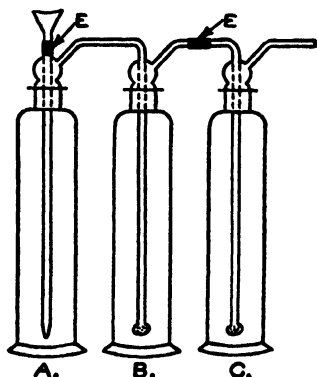
(1) If less  $\text{H}_2\text{SO}_4$  is required than is equivalent to half of the  $\text{Na}_2\text{CO}_3$  added, due to some of the  $\text{Na}_2\text{CO}_3$  reacting with soluble salts of  $\text{Ca}$  and  $\text{Mg}$ , soln originally contained no black alkali in excess, but rather an excess of so-called permanent or non-carbonate hardness. Express hardness in terms of  $\text{CaSO}_4$ . Difference between number of ml of the  $\text{H}_2\text{SO}_4$  required and half the number of ml of the  $\text{Na}_2\text{CO}_3$  added  $\times 0.00136$  = equivalent of  $\text{CaSO}_4$  in 100 ml of sample.

(2) If more  $\text{H}_2\text{SO}_4$  is required than that equivalent to half of the  $\text{Na}_2\text{CO}_3$  added, black alkali was originally present in the soln, and difference in ml  $\times 0.00106$  = black alkali in terms of  $\text{Na}_2\text{CO}_3$  in 100 ml of sample.



## BRINE

## BROMIDE IN PRESENCE OF CHLORIDE BUT NOT IODIDE (80)—TENTATIVE



A. REACTION CYLINDER.  
B & C. ABSORPTION CYLINDERS.  
E. RUBBER CONNECTIONS.

FIG. 58.—REACTION CYLINDER TO BE USED IN DETERMINATION OF BROMIDE

37.101

REAGENT

*Alkaline sodium sulfite soln.*—Dissolve 4 g of  $\text{Na}_2\text{SO}_3$  and 0.8 g of  $\text{Na}_2\text{CO}_3$  in  $\text{H}_2\text{O}$  and dilute to 100 ml.

37.102

APPARATUS

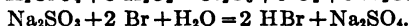
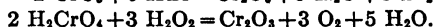
(a) *Reaction cylinder.*

(b) *Two high-form gas washing bottles.*

Join reacting cylinder and the two gas washing bottles as shown in Fig. 58.

37.103

REACTIONS



37.104

DETERMINATION

Take quantity of the brine that contains not more than 10 g of total salts. (Sample should not be too acid.) Evaporate to dryness or nearly so. Charge cylinder A by introducing glass beads to depth of ca 1", followed by 15 g of  $\text{CrO}_3$  crystals and finally enough glass beads to fill cylinder half full. Add 20 ml of the alkaline  $\text{Na}_2\text{SO}_3$  soln to cylinder B and 5 ml to cylinder C. Dilute each to ca 200 ml. Connect the three cylinders and draw current of air thru slowly. Wash sample into cylinder A with sufficient  $\text{H}_2\text{O}$  to make ca 25 ml of soln. Aspirate until contents of this cylinder are in soln and thoroly mixed, close inlet tube with small piece of rubber tubing and clamp, and reduce pressure in apparatus slightly by suction in order to guard against any possible escape of Br at ground-glass stopper. Allow to stand overnight and then aspirate with rather strong current of air (0.5–0.75 liter/min.) for 3 hours, adding four 2 ml portions of 3%  $\text{H}_2\text{O}_2$  soln to reaction flask at 30 min. intervals. Stop aspiration and evaporate contents of cylinders B and C nearly to dryness. Clean out cylinder A and freshly charge with glass beads and 15 g of  $\text{CrO}_3$  crystals.<sup>1</sup> To cylinder B add 10 g of KI crystals dissolved in 200 ml of  $\text{H}_2\text{O}$  and to C 3 or 4 g in a like quantity of  $\text{H}_2\text{O}$ . Connect apparatus, draw thru slow current of air, and transfer contents of evaporating dish to cylinder A by means of the small funnel, using 25 ml of  $\text{H}_2\text{O}$ . Aspirate until all the Br is evolved (ca 1 hour) and titrate the KI soln with standard 0.05 N  $\text{Na}_2\text{S}_2\text{O}_3$ . 1 ml of  $\text{Na}_2\text{S}_2\text{O}_3$  = 3.996 mg of Br.

## BROMIDE IN PRESENCE OF CHLORIDE AND IODIDE (81)—TENTATIVE

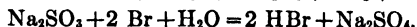
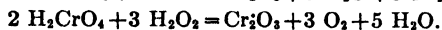
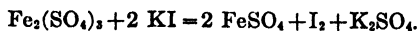
(Collaborative work indicates that following is the best method that has been published for determination of Br in presence of Cl and I, but results obtained show that only about 95% of the Br present is recovered when 80 mg of Br is contained in portion of sample taken for analysis. Method is satisfactory in absence of I.)

37.105

REAGENT AND APPARATUS.—See 37.101 and 37.102.

## 37.106

## REACTIONS



## 37.107

## DETERMINATION

Introduce 10 ml of sample into a distillation flask, adjust volume to ca 75 ml, and add 1.5–2.0 g of  $\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}$ . Distil off liberated I with steam, discarding distillate. Transfer residue from distillation flask to beaker, heat to boiling, add a few drops of methyl orange, and precipitate the Fe with  $\text{NH}_4\text{OH}$ , avoiding excess of  $\text{NH}_4\text{OH}$ , as a precipitate of  $\text{Ca}(\text{OH})_2$  is bulky and difficult to wash. Filter off the  $\text{Fe}(\text{OH})_3$ , wash with hot  $\text{H}_2\text{O}$ , and evaporate filtrate and washings to dryness or nearly so, taking care that during the evaporation the soln does not become acid from hydrolysis of  $\text{MgCl}_2$ . Proceed as directed under 37.104, beginning "Charge cylinder A."

## SALT (22)

## 37.108

## PREPARATION OF SAMPLE—TENTATIVE

If sample is coarser than 20 mesh, grind so that all will pass thru 20-mesh sieve, but avoid undue grinding so that as much as possible will be retained on an 80-mesh sieve. Mix sample by quartering and weigh all needed portions as nearly at the same time as possible.

## 37.109

## MOISTURE—TENTATIVE

Place ca 10 g of sample in dry, weighed Erlenmeyer flask of ca 200 ml capacity. Weigh flask and sample. Spread sample evenly over bottom of flask by shaking gently and insert small funnel in neck. Heat flask and sample for periods of 1 hour each on triangle over low, open flame of gas stove at ca  $250^\circ$  until two consecutive weighings agree within 5 mg. Shake flask occasionally so that sample will dry evenly. Report loss of weight as moisture.

## 37.110

## MATTERS INSOLUBLE IN WATER—TENTATIVE

Place 10 g of sample in 250 ml beaker, add 200 ml of  $\text{H}_2\text{O}$  at room temp., and let stand 30 min., stirring frequently. Filter thru weighed Gooch crucible with asbestos mat dried at  $110^\circ$ . Transfer residue to Gooch crucible with aid of policeman, using total of not more than 50 ml of  $\text{H}_2\text{O}$ . Wash residue with small portions of  $\text{H}_2\text{O}$ , ca 10 portions of 10 ml each, until 10 ml of filtrate shows only faint opalescence upon addition of a few drops of  $\text{AgNO}_3$  soln. Dry crucible and contents to constant weight at  $110^\circ$ . Call increase in weight of Gooch crucible, "matters insoluble in  $\text{H}_2\text{O}$ ," and report results in percentage on moisture-free basis. If matters insoluble in  $\text{H}_2\text{O}$  exceed 0.1%, determine their nature.

## 37.111

## MATTERS INSOLUBLE IN ACID (23)—TENTATIVE

Treat 10 g of sample with 200 ml of  $\text{HCl}$  (1+19), boil 2–3 min., and let stand 30 min., stirring frequently. Filter thru Gooch crucible with mat, dried at  $110^\circ$ . Express results in percentage.

**37.112 PREPARATION OF SOLUTION FOR SULFATE, CALCIUM, AND MAGNESIUM—TENTATIVE**

Weigh ca 20 g of sample, transfer to 400 ml beaker, and dissolve in 200 ml of HCl (1+3). Cover beaker, heat to boiling, and continue boiling gently for 10 min. Filter thru paper filter and wash residue with small quantities of hot H<sub>2</sub>O until filtrate is free from chlorides. Unite filtrate and washings, cool, and make to volume of 500 ml (soln A).

**37.113 SULFATE—TENTATIVE**

Place 250 ml of soln A, 37.112, in 400 ml beaker of resistant glass, heat to boiling, and add slight excess of hot 10% BaCl<sub>2</sub> soln dropwise while stirring. Concentrate by heating gently and finally evaporate to dryness on steam bath. Facilitate removal of free acid by stirring partly dried residue. Wash precipitate by decantation with small quantities of hot H<sub>2</sub>O, finally transferring precipitate to close-grained filter paper with aid of policeman and stream of hot H<sub>2</sub>O. Test filtrate for presence of Ba. Wash precipitate on filter until filtrate is free from chlorides. Dry and ignite filter containing precipitate over Bunsen flame. Report percentage of SO<sub>4</sub> in sample on moisture-free basis.

**37.114 CALCIUM—TENTATIVE**

Place remainder of soln A in 400 ml beaker of resistant glass. Add excess of 10% oxalic acid soln (10 ml usually will be sufficient). Add a few drops of methyl orange indicator, neutralize while hot by adding NH<sub>4</sub>OH dropwise, stirring constantly. Add ca 1 ml excess of the NH<sub>4</sub>OH, stir, and let stand in warm place for 3 hours. Decant supernatant liquid thru filter, reserving filtrate for determination of Mg. Test filtrate for Ca with NH<sub>4</sub> oxalate soln. Wash precipitate in beaker once with 10 ml of a 1% NH<sub>4</sub> oxalate soln, decanting thru filter paper. Combine filtrate and washings. Dissolve precipitate on filter with hot HCl (1+1), using same beaker, dilute to 100 ml, add a little more oxalic acid, and precipitate as before. After allowing to stand 3 hours, filter and wash with the NH<sub>4</sub> oxalate soln as before, reserving filtrate and washings. Transfer precipitate to crucible, dry, ignite, and heat over blast lamp to constant weight. Report as percentage of Ca on moisture-free basis.

**37.115 MAGNESIUM—TENTATIVE**

Combine filtrates and washings from the Ca determination, concentrate if necessary by boiling gently to volume of ca 150 ml, and proceed as directed under 37.64. Report as percentage of Mg on moisture-free basis.

**IODINE IN IODIZED SALT (24)—OFFICIAL****37.116 REAGENTS**

(a) *Bromine water*.—For alternative procedure, 37.118(b), determine approximate concentration (mg Br/ml) by adding (from buret) measured volume to flask containing 50 ml of H<sub>2</sub>O, 5 ml of 10% KI soln, and 5 ml of 1+9 H<sub>2</sub>SO<sub>4</sub> and titrating liberated I with 0.1 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>.

(b) *Sodium thiosulfate*.—0.005 N (recently standardized).

(c) *Starch soln*.—1% (freshly prepared). See 2.58(d).

(d) *Potassium iodide control soln*.—0.3280 g of reagent-grade KI/250 ml. Dilute 50 ml/250 ml, use 5 ml for control = 1.0 mg I and 1.308 mg KI.

## 37.117

## PREPARATION OF SAMPLE

Dissolve 50 g sample in  $H_2O$  and make to 250 ml in volumetric flask. Take 25 (for a) or 50 ml (for b) aliquots for analysis.

## 37.118

## DETERMINATION

(a) Place sample aliquot in 600 ml beaker and dilute to ca 300 ml. Neutralize to methyl orange with  $H_3PO_4$  and add 1 ml in excess. Proceed as directed under 27.57, third paragraph, starting "Add excess of Br water . . ."

(b) *Alternative procedure.*—Pipet 50 ml of sample soln into 200 ml Erlenmeyer flask. Neutralize to methyl orange with 2 normal  $H_2SO_4$ . Add Br water dropwise from buret in amount equivalent to 20 mg of Br. After a few minutes destroy greater portion of remaining free Br by adding 1%  $Na_2S_2O_3$  soln *dropwise while mixing*. Wash down neck and sides of flask with  $H_2O$  and complete removal of free Br by addition of 1 or 2 drops of 5% phenol soln. Add 1 ml of 2 normal  $H_2SO_4$  soln and 5 ml of 10% KI soln and titrate liberated I with the  $Na_2S_2O_3$  soln, adding 1 ml of the starch indicator near end of titration. Correct determination for blank on reagents and make one or more control determinations, using 50 ml of 20% reagent-grade NaCl soln to which is added appropriate quantities of the dilute control KI soln. 1 ml of 0.005  $N$   $Na_2S_2O_3$  = 0.1058 mg of I and 0.1384 mg of KI.

## 37.119

## METHOD OF REPORTING RESULTS—TENTATIVE

(In absence of added drying agents such as  $MgCO_3$ , Ca phosphate, etc.)

Convert the sulfate to  $CaSO_4$  and the unused Ca to  $CaCl_2$ , unless the sulfate in sample exceeds quantity necessary to combine with the Ca, in which case convert the Ca to  $CaSO_4$  and the unused sulfate first to  $MgSO_4$  and the remaining sulfate, if any, to  $Na_2SO_4$ . Convert unused Mg to  $MgCl_2$ . Add percentages of  $CaCl_2$  and  $MgCl_2$ . Report on moisture-free basis the percentage of matters insoluble in  $H_2O$ , of  $SO_4$ , of Ca, of Mg, of  $CaSO_4$ , of  $CaCl_2$ , and  $MgCl_2$ . Report also results of qualitative examination of matters insoluble in  $H_2O$ , if the quantity exceeds 0.1% on moisture-free basis.

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## 38. RADIOACTIVITY

38.1

### QUALITATIVE TEST—OFFICIAL

(Applicable to solids)

Charge an alpha ray electroscope (preferably of the Lind type) to bring leaf to suitable position on scale in microscope. Close door and record position of leaf on scale at frequent intervals, until rate of fall of leaf is constant. Calculate rate of fall of leaf in divisions per minute, designating figure obtained as natural leak of instrument for that particular determination.

Place convenient portion of sample on pan of the electroscope, close door, recharge leaf system, and record rate of fall of leaf in divisions/minute over same range of scale as before, until rate becomes constant, recharging if necessary. A rate of fall in excess of natural leak of instrument shows that sample is radioactive.

### QUANTITATIVE METHODS

#### *Emanation or Radon Method (1)—Official*

38.2

#### REAGENTS

(All reagents should be free from radium and radon)

- (a) *Nitrogen*.—Use tank N that has been allowed to stand at least 30 days.
- (b) *Standard radium soln*.—Use 100 ml standard soln containing  $10^{-9}$  g of Ra and 100 ml of blank rinsing soln issued by National Bureau of Standards.
- (c) *Resublimed  $P_2O_5$* .
- (d) *Calcium chloride*.—C.P. fused.
- (e) *Mercury*.—Redistilled.

38.3

#### APPARATUS

Use an all-glass apparatus (Fig. 59). Provide several units for storing sample (unit includes Nos. 2, 4, 5, 7, 8, 10, and 11).

Nos. 1 and 2 are 300 ml Pyrex flasks. Flask No. 1 has tube sealed on to introduce the standard soln. Nos. 3 and 4 are 8" condensers with ca  $\frac{3}{4}$ " inner tubes; 6 mm Pyrex tubing is used for all connections. No. 5 is a 24/40 joint; 7 and 10 are 7/25 joints; 8, 11, 17, 18, and 23 are 2 mm bore, well-ground, 2-way Pyrex stopcocks. Nos. 6, 9, 14, 16, and 21 are 2 mm bore, 3-way Pyrex stopcocks carefully lubricated with minimum of grease. Use care to keep bores free of grease. No. 12 is  $CaCl_2$  drying tube of  $\frac{3}{4}$ " Pyrex tubing 10" long having glass wool plug at each end. ( $P_2O_5$  may be substituted for the  $CaCl_2$ .) No. 13, a  $P_2O_5$  tube to remove last traces of  $H_2O$ , has same dimensions and construction as the  $CaCl_2$  tube, but is filled with mixture of glass beads and the  $P_2O_5$ . No. 15 is a Hg trap and bubble counter. Have center tube just dip below surface of the Hg. Use  $\frac{3}{4}$ " tubing with volume of ca 20 ml for each tube. No. 19 is closed-end manometer to measure vacuum of system. No. 20 is open-end manometer with ca 10 cm of Hg in each arm; it is used only near end of reflux to bring system to atmospheric pressure. No. 22 is an oil pump capable of producing vacuum of at least 0.5 mm. No. 24 is an ionization chamber, of ca 2.8 liters' capacity. It consists of a brass cylinder, 6" in height and diameter, provided with vacuum-tight brass inlet tube near top and brass outlet tube near bottom. Use care that no solder gets into chamber. Wall of the chamber should be grounded. The electrode is a  $\frac{1}{16}$ " brass wire, insulated from chamber by being threaded thru tapered amber plug and connected with a short lead to both the charging device and the leaf or fibers of the electroscope or electrometer. No. 25 may be either a

sensitive electroscope or an electrometer. Either the leaf- or filament-type electroscope may be used, or in the case of an electrometer the single- or double-filament type. To make electrical system as sensitive as possible, have the electroscope or electrometer mounted as close to the electrode as possible, preferably on top of ionization chamber. There should also be provided an electronic rectifying device capable of maintaining a potential of 200–500 volts. The negative terminal is connected by means of suitable switch to electrode of chamber and support of leaf or fibers of electroscope. The positive terminal is grounded.

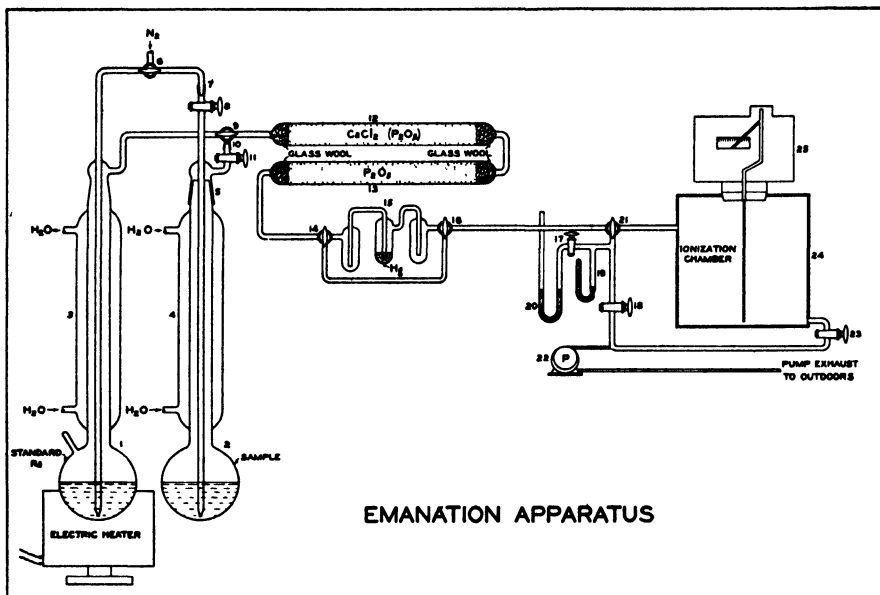


FIG. 59.—APPARATUS FOR DETERMINING RADIOACTIVITY BY EMANATION METHOD

### 38.4

#### PREPARATION OF SAMPLE

Run all samples in acid soln (either HCl or HNO<sub>3</sub>) and have them free from SO<sub>4</sub> and SiO<sub>2</sub>. Soln should be clear and limpid and contain no precipitate or suspended matter.

##### A. Samples completely soluble in acids:

(1) *Solid or semi-solid form.*—Add 50 ml of HNO<sub>3</sub> (1+9) and boil for several minutes. If a residue remains, add 50 ml of HCl (1+9) and again boil. (This treatment should not be applied to samples containing grease, such as face creams, the physical appearance of which will indicate that they are insoluble in aqueous solns.)

(2) *Liquid form (clear liquid, turbid liquid, or liquid containing suspended matter).*—Add 50 ml of HNO<sub>3</sub> (1+9) to 1–10 ml of sample, boil for several minutes, and examine carefully for opalescence. If a portion of the sample remains undissolved, add 50 ml of HCl (1+9) and again boil. If clear and limpid solns are obtained, proceed as directed under B (c).

**B. Samples wholly or partly insoluble in acids:**

**(a) Preliminary treatment:**

(1) *Solids*.—If sample is not in powder form, grind to fine powder; ignite weighed portion in porcelain dish in muffle at dull red heat, avoiding fusion; and proceed as directed under (b).

(2) *Semi-solids*.—Ignite quite rapidly in muffle a weighed portion of sample contained in porcelain dish, avoiding fusion. (Heating too slowly or heating in open may cause sample to creep over edge of dish.) Proceed as directed under (b).

(3) *Liquids immiscible with  $H_2O$* .—Evaporate weighed or measured portion of sample to dryness, or as nearly so as possible, on steam bath, and dry carefully on hot plate. Ignite residue in muffle, avoiding fusion. Proceed as directed under (b).

(4) *Liquids containing material insoluble in  $HNO_3$  (1+9)*.—Digest sample or suitable portion of it with  $HNO_3$  (1+9). Filter into 300 ml Florence flask, and wash residue thoroly with hot  $H_2O$ . Proceed as directed under (b), beginning "Ignite washed residue in Pt dish . . ."

**(b) Treatment of ash:**

(1) Digest ash obtained under (a) with  $HNO_3$  (1+9) on steam bath. Filter into Florence flask and wash thoroly with hot  $H_2O$ . (Flask of 300 ml capacity is usually most suitable, even if it is necessary to concentrate filtrates by boiling.) Ignite washed residue in Pt dish and cover residue with a few ml of  $H_2O$  and 5–10 ml of HF. Evaporate to dryness on steam bath. Add  $H_2O$  and a few ml of the  $HNO_3$ , digest on steam bath, filter into Florence flask, and wash with  $H_2O$ . Ash filter paper in Pt dish and add 5–10 ml of  $H_2O$  and 1 ml of the  $HNO_3$ . Examine carefully for any insoluble material; if none is found add soln directly to the Florence flask, rinsing dish several times with  $H_2O$  and adding washings to flask. Proceed as directed under (c).

(2) If an insoluble residue that does not contain  $BaSO_4$  remains, proceed as follows: Ignite insoluble residue in Pt dish and fuse with 5–10 times its weight of a fusion mixture consisting of equal weights of  $K_2CO_3$  and anhydrous  $Na_2CO_3$ . Cool, and cover with cover-glass. Neutralize fused mass with  $HNO_3$  (1+9), using a drop of phenolphthalein soln to note when soln is acid. Heat on steam bath, add a few ml excess of the  $HNO_3$ , and boil carefully. Filter soln into the Florence flask and wash thoroly. Ignite insoluble residue in Pt dish and proceed as directed under (b)(1), beginning "cover residue with a few ml of  $H_2O$  and 5–10 ml of HF."

(3) If insoluble residue contains appreciable quantities of  $BaSO_4$ , proceed as follows: Ignite insoluble residue in Pt crucible, mix, and fuse with 5–10 times its weight of a fusion mixture consisting of equal weights of  $K_2CO_3$  and anhydrous  $Na_2CO_3$ . Cool, boil residue with a little  $H_2O$  until thoroly disintegrated, and filter. Since this soln contains  $SO_4$  do not mix with the acid filtrate obtained under (b) (1). Wash residue with hot, normal  $Na_2CO_3$  until filtrate gives no test for  $SO_4$ , and then with a little  $H_2O$ . Dissolve washed residue ( $Ba$ - $RaCO_3$ ) carefully with  $HNO_3$  (1+1). If a clear soln results, combine with original acid filtrate. If an insoluble residue remains, proceed as directed under (b)(1), beginning "Ignite washed residue, etc." Combine with original acid filtrates.

**(c) Final preparation of clear solns:**

Evaporate the clear acid solns obtained under (a) or (b) to dryness in Pt dish. Add 10 ml of HCl (1+4) and again evaporate to dryness. Repeat the HCl evaporation.



Take up residue in 25 ml of the HCl, warm, and filter into 200 ml volumetric flask, washing dish and paper well with hot H<sub>2</sub>O until volume in flask is 100–125 ml. Add 40 ml of 10% BaCl<sub>2</sub> soln and make to volume. A clear limpid soln should result.

**38.5****DETERMINATION**

Keep stopcock (17) closed at all times except when system is at or near atmospheric pressure. With stopcocks 9 and 17 closed and 14, 16, and 21 open, evacuate system to pressure of ca 1 mm as shown on manometer 19. Close stopcock 18, shut off pump, and allow system to stand 1 hour. Pressure should remain less than 5 mm.

(1) *Introducing Standard.*—Place into flask 1, thru attached tube, the 10<sup>-9</sup> g Ra standard soln, 38.2(b). Rinse into flask with the blank soln.

(2) *Introducing Sample.*—Introduce into flask 2, a subdivision of the clear soln of sample obtained, (c), that will produce an accurately measurable increase in the rate of discharge of the electroscope, by disconnecting the flask and condenser at joint 5, and adjust to ca 200 ml with a boiled soln of 2% HCl containing 2% BaCl<sub>2</sub>. Then lubricate the joint with P<sub>2</sub>O<sub>5</sub> at its outer periphery, leaving joints 7 and 10 dry, and assemble apparatus, taking care to have the joints tight.

(3) *De-emanation of Standard.*—Manipulate stopcocks 6, 9, 14, 16, 18, and 21 so that gentle stream of N<sub>2</sub> (ca 3 bubbles/second as shown on the Hg trap) passing thru the soln in flask 1 will carry the Rn thru the pump exhaust to outdoors without entering the ionization chamber. Heat soln to boiling with electric heater and reflux 20–25 min. in gentle stream of N<sub>2</sub>. Remove electric heater, close cock 6 first and then 9, noting exact time that stopcock 9 is closed. This seals off the standard in flask 1, and as the soln cools the slight vacuum set up in this portion of the system minimizes loss of emanation.

(4) *De-emanation of Sample.*—Treat sample in flask 2 exactly as directed for the standard, taking care that stopcocks 6 and 9 are not turned, so that flask 1 is connected to the system during the refluxing of sample in flask 2. After sample has refluxed 20 min. close stopcocks 6 and 8 and then stopcock 11, and note exact time of closing latter. Thus the de-emanated sample is sealed in flask 2 by stopcocks 8 and 11. Then detach this portion of the apparatus at joints 7 and 10 and connect another empty sample flask and condenser unit on at joints 7 and 10.

(5) *Radon Accumulation.*—Allow both standard and sample to stand sealed 2–30 days. As may be seen in 44.31, ca 0.3 of the equilibrium quantity of Rn is formed in 2 days, 0.5 in 4 days, and practically complete equilibrium is reached in 30 days. Sensitivity of the determination is therefore to large degree controlled by period of storage of sample.

(6) *Background or Natural Leak.*—Connect electroscope and electrode with charging device so that negative potential of ca 300 volts (or potential necessary to set the leaf or fibers to maximum scale reading) is maintained on the electrode and the scope. Flush system by passing gentle stream of N<sub>2</sub> thru empty sample flask 2, the system, and ionization chamber for 30 min. Then close stopcock 8 and evacuate system to 1 mm pressure. Close stopcocks 18 and 23, shut off pump, and fill ionization chamber with N<sub>2</sub> at atmospheric pressure. Make final adjustment of pressure with manometer 20.

Remove source of negative potential from electrode and note time that leaf or fibers pass nearest division. Note exact time necessary for leaf or fibers to travel across one division and then allow system to stand until leaf or fibers travel across

major portion of scale, noting time necessary. To obtain natural leak, calculate rate of discharge of instrument in "divisions/minute."

(7) *Radon in Standard*.—Immediately after making final reading for natural leak determine Rn content of standard by transferring accumulated Rn in standard soln from flask 1 to ionization chamber as follows:

Evacuate ionization chamber and apparatus to stopcock 9, taking care that manometer 20 is shut off from system. Charge electroscope and electrode negatively and during refluxing maintain electrode at maximum negative potential, as indicated on electroscope. When system is evacuated to ca 1 mm of Hg, as indicated on manometer 19, manipulate stopcocks 6 and 9 very cautiously so that the Rn is carried in slow steam of  $N_2$  thru Hg bubble trap into evacuated chamber. Bring soln to boil and so adjust rate of  $N_2$  bubbling thru that at end of 20 min. refluxing pressure within ionization chamber is slightly less than atmospheric pressure, as indicated on manometer 20. (Gas velocity of the Rn- $N_2$  mixture during refluxing is 2–4 bubbles/second as indicated in Hg bubble trap and the  $N_2$  flow is 1–2 bubbles/second as shown in flask.) At end of 20 min. refluxing, remove heat under flask 1 while  $N_2$  is still bubbling thru the standard soln. As soon as pressure in ionization chamber is equal to that of atmosphere, shown on manometer 20, close stopcock 6 and then 9 to seal this part of system off again to allow another accumulation of Rn for future standardizations, and note exact time of closing stopcock 9.

Allow the mixture of Rn and  $N_2$  in ionization chamber to stand 3 hours to allow the Rn to form equilibrium quantities of radium A, B, and C before taking readings. Remove source of negative potential from electrode and electroscope exactly 3 hours after stopcock 9 is closed, and take readings of electroscope with aid of stop-watch over same range as was used for natural leak determination.

After completing the readings, pump out ionization chamber and rinse 2 or 3 times with pure  $N_2$  by evacuating and refilling ionization chamber, passing the  $N_2$  thru empty sample flask 2.

(8) *Redetermination of Natural Leak*.—Allow system to stand at least 3 hours and redetermine the background of electroscope as directed previously.

(9) *Radon in Sample*.—Disconnect empty sample flask 2 at joints 7 and 10 and replace with sealed flask 2, which contains the sample, after lubricating joint 10 with  $P_2O_5$ . For transfer of Rn from sample to ionization chamber and measurement of ionization, proceed as directed under (7).

(10) *Calculations*.—Subtract natural leak of electroscope in terms of divisions/minute from rate of fall in divisions/minute when the Rn from standard soln was in the chamber. In sample sealed for less than 30 days calculate Rn content of standard from 44.31. In sample sealed for 30 days or more, Rn content is substantially equivalent to Ra content. Divide calculated quantity of Rn by acceleration of rate of discharge of electroscope due to this amount of Rn. Quotient will be millimicrograms of Ra equivalent to an acceleration of one division/minute in rate of discharge of electroscope.

Subtract natural leak of electroscope in divisions/minute from accelerated rate of fall due to Rn of sample. Multiply this difference, which is net effect of the Rn alone in sample, by the millimicrograms of Ra that will cause an increase of 1 division/minute as found in the standardization. If sample has been allowed to stand 30 days, result will be quantity of Ra in subsample taken for analysis. If sample has stood less than 30 days, calculate the Ra content from 44.31. Report result in millimicrograms of Ra/ml or/g.

*Gamma Ray Method Using Electroscope (2)—Tentative*

## 38.6

## APPARATUS

A cylindrical metal chamber (1) of ca 1000 ml capacity, which is hermetically sealed. Axis of cylinder is vertical. On the inside is the Wulf 2-fiber system, which is fastened to an amber insulator (4), and which can be charged with aid of electronic rectifier charging device or suitable charging rod. Rate of movement of the fibers is determined by means of a microscope (2). (See Fig. 60.)

## 38.7

## PREPARATION OF SAMPLE

(a) *Effective radioactivity*.—In the case of devices and preparations in which radioactive material is not ingested, but applied externally, determine the effective radioactivity directly upon sample without removing it from container in which it is to be used.

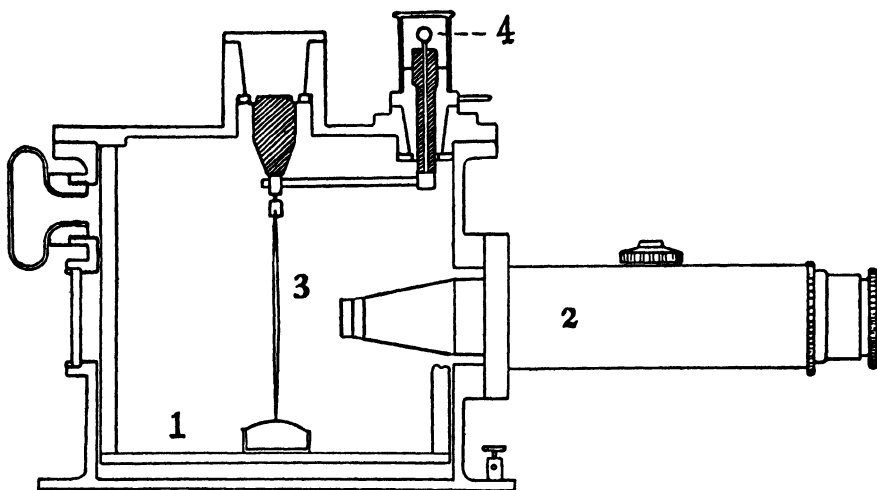


FIG. 60.—GAMMA RAY ELECTROSCOPE

(b) *Total radioactivity*.—Hermetically seal entire sample or one or more subdivisions in suitable container, such as test tube or flask. Allow to stand at least 30 days.

## 38.8

## STANDARDS

Use known quantities of Ra measured by National Bureau of Standards.

## 38.9

## STANDARDIZATION OF ELECTROSCOPE

(a) *Natural leak*.—Charge electroscope thru charging rod by means of charging device, to bring fibers to suitable point, for example at 70° division mark, after charging rod (4) is grounded. As natural leak of the electroscope in a room free from Ra is very small, use a Ra standard to adjust fiber approximately to desired division mark. Remove standard from room and record time when fiber crosses exact division mark. Allow electroscope to remain charged overnight. Again record time when one of the fibers crosses an exact division mark. Calculate rate of travel of fiber in seconds per division and designate figure obtained as natural leak (R) of electroscope for the particular determination.

(b) *Constant*.—Place a suitable Ra standard containing 10–1000 micrograms of Ra at an exact measured distance from center of electroscope. Charge electroscope and record average time, measured by stop-watch, for at least 6 trials, of fiber to travel over that part of scale used in obtaining the natural leak. Calculate rate of travel in seconds per division and corrected time (T) due to Ra alone by following formula:

(1)  $T = PQ/(Q - P)$ , in which P = observed time and Q = natural leak.

Then calculate the constant (K) by following formula:

(2)  $K = ST/(R)^2$ , in which S = micrograms of Ra in standard; T = corrected time found in (1); and R = distance between center of electroscope and standard.

To obtain a reliable average figure for this constant, calculate K, placing the Ra standard at different distances from center of electroscope. Use several different standards of known Ra content.

## 38.10

## DETERMINATION

Place sample at suitable distance from center of electroscope. Charge electroscope as directed above, using if convenient a Ra standard to adjust fiber. Record average time taken by fiber to travel between exact division marks over that part of the scale used for the standardization. If sample contains sufficient radioactivity to permit, take average readings when it is placed at different distances from center of electroscope; if it contains only a relatively small quantity of radioactivity, fasten it with rubber bands to circumference of electroscope so as to obtain maximum ionization. Calculate micrograms of Ra (S') or its equivalent in terms of Ra by following formula:

(3)  $S' = K(R')^2/T'$ , in which K = constant of electroscope; R' = exact distance between center of electroscope and center of sample; and T' = corrected time in seconds per division due to radioactivity only in sample.

*Gamma Ray Method Using Geiger-Müller Counter (s)—  
Official, First Action*

## 38.11

## APPARATUS

A Geiger-Müller discharge counter of the coaxial type and an appropriate electrical circuit for this counter. Response of instrument to gamma ray should be linear to at least 10,000 counts/minute and sensitivity such that 0.1 microgram of Ra will at least double the background count.

## 38.12

## STANDARDS

Set of 13 gamma-ray standards prepared and measured by National Bureau of Standards, ranging in value from 0.1 to 100 micrograms of Ra, in the following steps: 0.1, 0.2, 0.2, 0.5, 1.0, 2.0, 2.0, 5.0, 10.0, 20.0, 20.0, 50.0, 100.

## 38.13

## PREPARATION OF SAMPLE.—See 38.7(a) and (b)

## 38.14

## DETERMINATION

Turn instrument on and allow to stand at least 15 min. before determination is started.

1. Determine background count.

2. Place sample at such a distance from counter that indication on meter may be conveniently read. Roughly estimating value of this reading, remove sample and put in its place Ra standard of such strength that about same reading will be obtained.

3. Remove sample and standard to where they will not affect the background and wait 5 min. for background to become stable.

4. Make comparison of sample against standard, placing each in same relative position to counter. Take check background readings between each observation and allow sufficient time for instrument to come to equilibrium for each reading of sample, standard, and background. This equilibrium time will be dependent on the time constant of the instrument and may be from 1 to 5 min. or even longer. If individual counts are recorded, total count over each period is sufficient. If meter readings are recorded, make these readings at 30-second intervals during run and record the average. Make two check determinations.

Typical schedule:

	Time (min.)	Reading
1	0	Background run starts
	5	Background run ends
	Place sample in position	
2	10	Sample run starts
	15	Sample run ends
	Sample removed	
3	20	Background run starts
	25	Background run ends
	Place standard in position	
4	30	Standard run starts
	35	Standard run ends
	Remove standard	
5	40	Background run starts
	45	Background run ends

### 38.15

#### CALCULATION

1. Average each successive pair of background readings and subtract this average background reading from appropriate sample reading and standard reading: i.e., in example given above average 1 and 3 and subtract this average from 2, and then average 3 and 5 and subtract from 4.

2. From the 3 comparisons 6 independent ratios of sample to standard are obtained.

3. Calculate probable error of measurement according to formula:

$$\text{Probable error} = \pm 0.67 \sqrt{\frac{\sum(\bar{x} - x)^2}{n(n-1)}}, \text{ in which}$$

$\bar{x}$  = average ratios of sample to standard;  $x$  = each individual ratio of sample to standard;  $n$  = number of ratios; and  $\sum$  = summation sign.

4. Multiply value of standard by average ratio and express result as micrograms ( $\text{g} \times 10^{-6}$ ) of Ra  $\pm$  probable error (also in micrograms of Ra). This is the radioactivity of subdivision used for the analysis.

5. Express final result in millimicrograms ( $\text{g} \times 10^{-9}$ ) or micrograms ( $\text{g} \times 10^{-6}$ ) of Ra/g or/ml  $\pm$  probable error, multiplying or dividing the "probable error" by same factor that is used for conversion of the radioactivity of the subdivision.

#### SELECTED REFERENCES

- (1) Rev. Sci. Instruments, **4**, 216 (1933); **6**, 99 (1935); Phy. Rev., **55**, 931 (1939).
- (2) J. Assoc. Official Agr. Chem., **19**, 101 (1936).
- (3) Ibid., **25**, 103, 618 (1942).

## 39. DRUGS

### SAMPLING (1)—OFFICIAL, FIRST ACTION

#### 39.1

##### *I. Tablets and Pills*

(a) *Bulk lots*.—Mix the lot as thoroly as possible without mutilating the contents. Count, weigh, and powder thoroly at least 100 units. Calculate average weight per unit.

(b) *Containers of 1000 or more units*.—Open and cautiously mix entire contents without mutilation and divide into 2 parts. Take one part (usually  $\frac{1}{2}$  or  $\frac{1}{3}$  of sample is sufficient) for analysis. Return remainder to container as reserve sample. Count, weigh, and powder analyst's subdivision as directed under (a).

(c) *Containers of 100–500 units*.—If more than one container is available, count, weigh, and powder entire contents of one of them. If only one container is available, but there is sufficient material to warrant subdividing, proceed as directed under (b); otherwise count, weigh, and powder entire contents.

(d) *Small containers, e.g., tubes of hypodermic tablets*.—Choose a number of containers that will constitute a satisfactory sample; count, weigh, and powder contents.

(e) *Tablets or pills of small dosages, e.g., 1/100 grain of active ingredient*.—The number of units necessary may be so large as to make powdering unnecessary. A half or whole bottleful may be required. Count the units to be used but do not powder them.

##### *II. Soft Capsules*

Count and weigh a representative number of capsules and ascertain gross weight per capsule. Open capsules and transfer as much of contents as possible to weighing bottle. Clean capsules (cutting in two if necessary) and wash by agitating with alternate portions of alcohol and ether. (A few drops of acetic acid mixed with the alcohol aids in the cleaning.) Finally remove the ether before a fan or air blast. Deduct weight of cleaned, empty capsules from gross weight and calculate average net contents.

##### *III. Ampuls*

Before opening ampuls dislodge any liquid adhering in neck. Mark with file or other suitable instrument the level of the liquid on the necks of requisite number of ampuls, open them near the tip, transfer bulk of contents to small flask, and mix. To determine volume of contents, wash and dry empty ampuls and fill to mark with  $H_2O$  from a graduated pipet or buret.

### ACETANILID AND ACETOPHENETIDIN (2)—(PHENACETIN)

#### 39.2

##### *Qualitative Test for Acetophenetidin—Tentative*

To 0.001–0.002 g of sample in test tube add a drop of acetic acid, 0.5 ml of  $H_2O$ , and 1 ml of 0.1 N I; warm mixture to ca  $40^\circ$  and add a drop of HCl. If acetophenetidin alone is present, its periodide separates almost immediately in the form of reddish brown leaflets or needle-like crystals. If sample consists largely of acetanilid, separation takes place on cooling and shaking the liquid. In the presence of considerable acetanilid, the periodide first separates as minute, oily globules, which on vigorous shaking gradually become crystalline. By this test as little as 0.0005 g of acetophenetidin, if alone, may be detected in the form of its characteristic periodide.

*Quantitative Methods—Tentative*

## 39.3

## REAGENTS

(a) *Purified iodine*.—Use reagent quality I.

(b) *Standard sodium thiosulfate soln.*—Dissolve 30 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in recently boiled, cooled  $\text{H}_2\text{O}$  and dilute to ca 1 liter. Standardize this soln against the reagent I as follows: Weigh accurately ca 0.3 g of the I in small glass capsule provided with closely fitting glass cap or stopper. Place capsule in 200 ml Erlenmeyer flask containing 0.5 g of KI dissolved in 1–2 ml of  $\text{H}_2\text{O}$ . After complete soln dilute with 10 ml of  $\text{H}_2\text{O}$  and titrate with the  $\text{Na}_2\text{S}_2\text{O}_3$  soln, using 1 or 2 drops of starch indicator, 6.3(e).

(c) *Standard iodine soln.*—Dissolve 40 g of KI in least possible quantity of  $\text{H}_2\text{O}$ , add 30 g of I, and after soln dilute to ca 1 liter. Standardize against the standard  $\text{Na}_2\text{S}_2\text{O}_3$  soln.

## 39.4

## DETERMINATION

(a) *Acetophenetidin* (1) Volumetric.—Place 0.2 g of the acetanilid-acetophenetidin mixture in a 50 ml lipped Erlenmeyer flask, add 2 ml of acetic acid, heat gently over wire gauze to complete soln, and dilute with 40 ml of  $\text{H}_2\text{O}$  previously warmed to  $70^\circ$ . Transfer clear liquid with two 10 ml portions of warm ( $40^\circ$ )  $\text{H}_2\text{O}$  to glass-stoppered, 100 ml volumetric flask containing 25 ml of the standard I soln warmed to  $40^\circ$ . Stopper, mix thoroly by rotating liquid, add 3 ml of HCl, continue rotating liquid until crystallization begins, and then set aside to cool. (If ratio of acetophenetidin to acetanilid is equal to or greater than unity, crystalline scales will form almost immediately on addition of acid. As proportion of acetanilid increases, however, periodide tends to remain in liquid state. Gentle agitation or rotation of flask in  $\text{H}_2\text{O}$ , warmed not to exceed  $40^\circ$ , hastens formation of crystals.) When contents are at room temp., fill flask with  $\text{H}_2\text{O}$  to within 2 or 3 ml of mark, mix thoroly by rotating mixture, and allow to stand overnight. Fill to mark with  $\text{H}_2\text{O}$ , mix thoroly, allow to stand 30 min., and filter thru a 5.5 cm dry, closely fitted filter into 50 ml volumetric flask, rejecting ca 15 ml of first runnings but reserving it for recovery of acetanilid. Transfer the 50 ml aliquot to 200 ml Erlenmeyer flask and titrate excess I with the standard  $\text{Na}_2\text{S}_2\text{O}_3$  soln. The formula of the precipitated periodide is  $(\text{C}_7\text{H}_5\text{O} \cdot \text{C}_6\text{H}_4\text{NH} \cdot \text{COCH}_3)_2\text{HI} \cdot \text{I}_4$ . 1 ml of 0.25 N I = 0.0224 g of  $\text{C}_{10}\text{H}_{13}\text{O}_2\text{N}$ .

(2) Gravimetric.—Filter off the periodide, preferably by suction; wash with 10–15 ml of the standard I soln; and transfer precipitate, together with filter and any particles of precipitate remaining in volumetric flask, to separator, using not over 50 ml of  $\text{H}_2\text{O}$ . Remove both free and added I with a few small crystals of  $\text{Na}_2\text{SO}_3$ , and extract liquid with three 50 ml portions of  $\text{CHCl}_3$ , washing each portion subsequently in second separator with 5 ml of  $\text{H}_2\text{O}$ . After washing and clearing, filter the  $\text{CHCl}_3$  soln thru small dry filter into 200 ml Erlenmeyer flask, distil most of the  $\text{CHCl}_3$ , transfer residual soln (5–10 ml) by means of a little  $\text{CHCl}_3$  to small weighed beaker, evaporate to dryness on steam bath, cool, and weigh.

(b) *Acetanilid*.—If combined weight of the acetanilid-acetophenetidin mixture is known, determine weight of acetanilid by difference; or determine it directly from a second aliquot of filtrate from the acetophenetidin periodide (a) as follows:

Pipet 25–30 ml of the clear liquid into separator, decolorize with solid  $\text{Na}_2\text{SO}_3$ , and add solid  $\text{NaHCO}_3$  in slight excess, then 1 or 2 drops of acetic anhydride. Extract with three 60 ml portions of  $\text{CHCl}_3$ , passing the  $\text{CHCl}_3$  soln thru small, dry filter into 200 ml Erlenmeyer flask, and distil the  $\text{CHCl}_3$  by the aid of gentle heat to ca 20 ml. Add 10 ml of  $\text{H}_2\text{SO}_4$  (1 + 9) and digest on steam bath until residue has been reduced one-half. Add 20 ml of  $\text{H}_2\text{O}$  and continue digestion for an hour. Add a

second 20 ml portion of  $\text{H}_2\text{O}$  and 10 ml of  $\text{HCl}$  and titrate very slowly, dropwise, with standard bromide-bromate soln, 39.5(a), until a faint yellow color remains. While adding this reagent, rotate flask sufficiently to agglomerate the precipitated tribromoaniline. 1 ml of 0.1  $N$   $\text{KBr-KBrO}_3 = 0.00225 \text{ g of } \text{C}_6\text{H}_5\text{ON}$ .

If the preparation contains antipyrine or caffeine, or both, in addition to acetanilid and acetophenetidin, proceed as follows: (1) Digest mixture by heating with the  $\text{H}_2\text{SO}_4$  (1+9) to convert acetophenetidin and acetanilid to phenetidin and aniline sulfates, respectively, 39.7(a); (2) remove caffeine and antipyrine by extraction with  $\text{CHCl}_3$  and separate them as in 39.47; (3) regenerate acetophenetidin and acetanilid by treating the soln of the corresponding sulfates with solid  $\text{NaHCO}_3$  in slight excess and a few drops of acetic anhydride, and extract with  $\text{CHCl}_3$ . Evaporate the  $\text{CHCl}_3$ , dry, and weigh the residue. Separate the constituents as in simple mixtures.

#### ACETANILID AND CAFFEINE (3)—OFFICIAL

##### 39.5

##### REAGENTS

(a) *Standard bromide-bromate soln.*—Dissolve 14 g of  $\text{KBrO}_3$  and 55 g of  $\text{KBr}$  in  $\text{H}_2\text{O}$ . Dilute to 1 liter and standardize against recrystallized and dried acetanilid, by one of following procedures: (1) Proceed as directed under 39.4(b), beginning "Add 10 ml of  $\text{H}_2\text{SO}_4$  (1+9)"; (2) transfer 10 ml of the soln to glass-stoppered flask and add 25 ml of  $\text{H}_2\text{O}$ , 5 ml of 16.5%  $\text{KI}$  soln, and 5 ml of  $\text{HCl}$ . Shake thoroly and titrate the liberated  $\text{I}$  with 0.1  $N$   $\text{Na}_2\text{S}_2\text{O}_3$ , using starch soln as indicator, 6.3(e).

(b) *Iodine soln.*—Dissolve 2 g of  $\text{I}$  and 6 g of  $\text{KI}$  in  $\text{H}_2\text{O}$  and dilute to 100 ml.

Treat all corks used in the distillation with  $\text{CHCl}_3$ .

##### 39.6

##### PREPARATION OF SOLUTION

(a) Weigh 0.3–0.5 g of powdered sample or, if preferred, a quantity equal to, or multiple of, average unit dose (previously ascertained by weighing collectively 20 or more such doses). Transfer to separator, add 50 ml of  $\text{CHCl}_3$  and 20 ml of  $\text{H}_2\text{O}$ , shake vigorously, and after clearing draw off lower layer thru small dry filter into 300 ml Erlenmeyer flask. Repeat extraction twice, using 50 ml portions of  $\text{CHCl}_3$  for each extraction. Recover any caffeine-acetanilid mixture observable about apex of delivery tube of separator, edge of filter, and tip of separator by careful washing with  $\text{CHCl}_3$ , and add these washings to main portion. Distil combined  $\text{CHCl}_3$  extracts to ca 10 ml.

If caffeine is present, as free alkaloid or in other readily extractable form, extraction may, if preferred, be made on filter paper by washing with successive 5–10 ml portions of  $\text{CHCl}_3$  (30–50 ml is usually sufficient) until extraction is complete, as indicated by absence of any residue after evaporation of small portion of last washing.

(b) With dilute alcoholic solns, evaporate a measured quantity on steam bath until most of alcohol has been expelled, or take an aliquot of residue from an alcohol determination and transfer to separator by pouring and rinsing with minimum quantity of  $\text{H}_2\text{O}$  so that final volume does not greatly exceed 20 ml. In order to avoid any loss of acetanilid by hydrolysis during evaporation, add a little solid  $\text{NaHCO}_3$  and a drop of acetic anhydride. Should preparation contain other alkaloids, acidify with a few drops of  $\text{H}_2\text{SO}_4$  (1+9) immediately after acetylation to retain such basic material in the aqueous soln. Add 50 ml of  $\text{CHCl}_3$ , shake vigorously, and after clearing draw off the  $\text{CHCl}_3$  layer thru a filter into 200 ml Erlenmeyer flask. Repeat extraction twice, using 50 ml portions of  $\text{CHCl}_3$  for each extraction, and distil combined  $\text{CHCl}_3$  washings to volume of ca 10 ml.



## 39.7

## DETERMINATION

(a) *Caffeine*.—Treat the  $\text{CHCl}_3$  soln obtained, 39.6, with 10 ml of  $\text{H}_2\text{SO}_4$  (1+9) and digest on steam bath until contents of the flask are reduced to 5 ml. Add 10 ml of  $\text{H}_2\text{O}$  and continue digestion until liquid is again reduced to 5 ml. (Diluting and evaporating must be repeated until odor of acetic acid can no longer be detected in vapors.) Cool, and transfer to separator with minimum of  $\text{H}_2\text{O}$ . (Final volume should not greatly exceed 20 ml.) Add 50 ml of  $\text{CHCl}_3$ , extract in usual way, and after clearing withdraw lower layer thru small, dry filter into 200 ml Erlenmeyer flask. Repeat extraction with two 50 ml portions of  $\text{CHCl}_3$ . Distil combined extracts to ca 10 ml, finally transferring residual liquid, by washing with  $\text{CHCl}_3$ , to weighed beaker or crystallizing dish. Allow soln to evaporate spontaneously, or by gentle heat and an air blast, to apparent dryness. Cool, and allow to stand in open until weight becomes constant.

From preparations containing powdered cinnamon, celery seed, ginger, or other vegetable products,  $\text{CHCl}_3$  extracts, in addition to caffeine and acetanilid, certain oils, fats, waxes, resins, pigments, and other substances. After the caffeine-acetanilid mixture has been digested, these oils, etc., appear either in suspension or soln and contaminate the caffeine. Remove any suspended impurities by filtering thru small, moistened filter immediately after hydrolysis and prior to extraction with  $\text{CHCl}_3$ . Should recovered caffeine be deeply colored or contaminated with foreign matter, purify as follows: Dissolve in  $\text{H}_2\text{SO}_4$  (ca 5 ml of 0.2 *N* acid for every 100 mg of caffeine); filter, if necessary, thru moistened filter; add 1 ml of 9 *N*  $\text{H}_2\text{SO}_4$  and sufficient iodine reagent, 39.5(b), to color supernatant liquid a deep claret; stir, and allow to stand an hour, preferably in refrigerator. Filter and wash the periodide with a few ml of *I* soln; transfer both filter and precipitate to separator, using not more than 20 ml of  $\text{H}_2\text{O}$ ; and decolorize with a crystal of  $\text{Na}_2\text{SO}_3$ . Extract with three 50 ml portions of  $\text{CHCl}_3$  and proceed as directed above.

(b) *Acetanilid*.—(1) Transfer the soln of aniline sulfate remaining in separator to the Erlenmeyer flask used in effecting hydrolysis and heat 10 min. on steam bath to expel all traces of  $\text{CHCl}_3$ . Wash filter that was used in drying the  $\text{CHCl}_3$  soln of caffeine with 5 ml of  $\text{H}_2\text{O}$ , adding washings to main soln of aniline sulfate. Add 10 ml of  $\text{HCl}$  and titrate with the standard bromide-bromate soln, 39.5(a), until a faint yellow coloration remains, rotating flask sufficiently to agglomerate the precipitated tribromoaniline. 1 ml of 0.1 *N*  $\text{KBr-KBrO}_3 = 0.00225$  g of  $\text{C}_6\text{H}_5\text{ON}$ .

(2) Add an excess of the standard bromide-bromate soln to soln of aniline sulfate obtained under (b) and titrate the excess with 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3$  after addition of 5 ml of *KI* soln and starch soln as indicator, 6.3(e). 1 ml of 0.1 *N* bromide-bromate = 0.00225 g of acetanilid.

(c) *Other ingredients*.—To determine  $\text{NaHCO}_3$  also, which often appears as the  $\text{CHCl}_3$ -insoluble residue, titrate such residue with standard acid, using methyl orange indicator. The bicarbonate may also be determined by igniting the original sample (if talc is absent) or the  $\text{CHCl}_3$ -insoluble residue, with  $\text{H}_2\text{SO}_4$  and weighing resulting  $\text{Na}_2\text{SO}_4$ .  $\text{Na}_2\text{SO}_4 \times 1.183 = \text{NaHCO}_3$ .

Should the "acetanilid compound" be combined with  $\text{NaBr}$ , the  $\text{Br}$ , in the absence of other halides, may be determined volumetrically as directed under 12.44. 1 ml of 0.1 *N*  $\text{AgNO}_3 = 0.01029$  g of  $\text{NaBr}$ .

## ACETANILID, CAFFEINE, AND CODEINE (4)—OFFICIAL

## 39.8

## REAGENTS

(a) *Iodine soln*.—See 39.5(b).

(b) *Methyl red indicator*.—Dissolve 0.1 g of methyl red in 100 ml of neutralized alcohol and filter if necessary.

## 39.9

## PREPARATION OF SOLUTION

Transfer to separator one or more average unit doses (ca 0.2 g of acetanilid) of the powdered sample; add 20 ml of  $H_2O$ , 50 ml of  $CHCl_3$ , and 10 drops of  $H_2SO_4$  (1+9); and extract in usual way. After clearing, wash solvent in second separator with 5 ml of  $H_2O$  and transfer to 200 ml Erlenmeyer flask. Repeat extraction with two 50 ml portions of  $CHCl_3$ , finally distilling combined  $CHCl_3$  solns by gentle heat to ca 10 ml. Test for complete extraction.

## 39.10

## DETERMINATION

(a) *Acetanilid and caffeine*.—Treat the  $CHCl_3$  residue, 39.9, as directed under 39.7.

(b) *Codeine*.—Combine the wash  $H_2O$  used in second separator under 39.9 with the soln of codeine sulfate. Add an excess of solid  $NaHCO_3$ , extract with 5 successive portions of 30, 25, 20, 15, and 10 ml of  $CHCl_3$ , wash the combined  $CHCl_3$  extracts with 5 ml of  $H_2O$  in second separator, and pass thru dry filter into 200 ml Erlenmeyer flask. Test for complete extraction, evaporate to apparent dryness in a small weighed beaker on steam bath, add a few drops of alcohol and like quantity of  $H_2O$  to amorphous residue, and evaporate again. Finally cool and allow the usually crystalline product to stand until weight becomes constant. Check this result volumetrically by dissolving residue in 3–5 ml of neutral alcohol and titrating with 0.02  $N$   $H_2SO_4$  to faint red color, using methyl red indicator, 39.8(b). 1 ml of 0.02  $N$   $H_2SO_4$  = 0.00599 g of  $C_{18}H_{21}O_3N$ , or 0.00635 g of  $C_{18}H_{21}O_3N \cdot H_2O$ .

The quantity of codeine found by weight will usually be slightly greater than that determined by titration. To insure greatest possible accuracy in volumetric operations, check strength of the standard acid used by titration against pure codeine.

## ACETANILID, CAFFEINE, AND QUININE (5)—OFFICIAL

## 39.11

## REAGENT

*Bromocresol purple soln*.—Triturate 0.1 g of bromocresol purple in agate mortar with 9 ml of 0.02  $N$   $NaOH$ . After soln dilute with  $H_2O$  to 200 ml, and filter if necessary. Soln should be deep orange to red in color. If it is purple, addition of not more than 0.5 ml of 0.02  $N$  acid should make it red. If it is yellow, addition of not more than 0.5 ml of 0.02  $N$  alkali should produce the red color.

## 39.12

## PREPARATION OF SOLUTION.—See 39.9

## 39.13

## DETERMINATION

(a) *Acetanilid and caffeine*.—See 39.7.

(b) *Quinine*.—Combine the wash  $H_2O$  used in second separator under 39.9 with the soln of quinine bisulfate, add a slight excess of  $NH_4OH$ , and extract with three 50 ml portions of  $CHCl_3$ . Wash each portion with 5 ml of  $H_2O$  in second separator and pass thru dry filter into 200 ml Erlenmeyer flask. Evaporate on steam bath to apparent dryness, dissolve the amorphous alkaloid in 5 ml of neutral alcohol, and titrate with 0.02  $N$   $H_2SO_4$  to yellow color, using 2 drops of bromocresol purple indicator, 39.11. Heat on steam bath until most of alcohol has been expelled, adding, if necessary, sufficient 0.02  $N$   $H_2SO_4$  to maintain the acid reaction. 1 ml of 0.02  $N$   $H_2SO_4$  = 0.00757 g of quinine ( $C_{20}H_{24}O_2N_2 \cdot 3H_2O$ ) or 0.00783 g of quinine sulfate,

$(C_{20}H_{24}O_2N_2)_2 \cdot H_2SO_4 \cdot 2H_2O$ , or 0.00793 g of quinine hydrochloride,  $C_{20}H_{24}O_2N_2HCl \cdot 2H_2O$ , or 0.00795 g of quinine dihydrochloride,  $C_{20}H_{24}O_2N_2 \cdot 2HCl$ .

If the mixture contains acetophenetidin in place of acetanilid, proceed as outlined above, except to make the separation of caffeine and acetophenetidin as directed under 39.19.

#### ACETANILID, CAFFEINE, QUININE, AND MORPHINE (5)—OFFICIAL

##### 39.14

##### PREPARATION OF SOLUTION

Transfer to separator a quantity of the powdered sample containing not less than 0.016 g of morphine, and add 20 ml of  $H_2O$  and 10 drops of  $H_2SO_4$  (1 + 9). Extract with three 50 ml portions of alcohol-free  $CHCl_3$ , wash each portion in a second separator with 5 ml of  $H_2O$ , and add combined washings to the alkaloidal soln in first separator. Filter the  $CHCl_3$  extracts thru small, dry filter into a 200 ml Erlenmeyer flask and distil by gentle heat to ca 10 ml.

##### 39.15

##### DETERMINATION

(a) *Acetanilid and caffeine*.—Proceed as directed under 39.7. using the  $CHCl_3$  extract obtained under 39.14.

(b) *Quinine*.—Add to the soln of quinine and morphine sulfates obtained under 39.14, 4–5 ml of 10%  $NaOH$  soln and extract with four 40 ml portions of  $CHCl_3$ . Wash each portion with 5 ml of  $H_2O$  and pass the clear solvent thru small, dry filter into 200 ml Erlenmeyer flask. Remove solvent by gentle distillation and titrate residual quinine with 0.02  $N$   $H_2SO_4$  as directed under 39.13(b). (If the morphine salt present is contaminated with codeine, the latter will be separated and titrated with the quinine.)

(c) *Morphine*.—Wash filter used under (b) with 5 ml of  $H_2O$  and add washings to the aqueous, alkaline soln of the alkaloid. Add 0.5 g of  $NH_4Cl$  (or a quantity slightly in excess of that required to free the morphine as well as convert all  $NaOH$  to  $NaCl$ ), 45 ml of  $CHCl_3$ , and 5 ml of alcohol. Extract in usual way, washing solvent in second separator with 5 ml of  $H_2O$ . Pass the  $CHCl_3$  thru small, dry filter into 200 ml Erlenmeyer flask. Repeat extraction with three 40 ml portions of  $CHCl_3$ , washing and filtering as before. Collect all solvent in Erlenmeyer flask and distil to ca 10 ml. Transfer with  $CHCl_3$  to small beaker and evaporate to apparent dryness. Dissolve residue in 1–2 ml of warm, neutral methyl alcohol; add a drop of the methyl red indicator, 39.8(b), and titrate with 0.02  $N$   $H_2SO_4$  to faint red color. Evaporate most of the alcohol on steam bath and, if necessary, add from a buret sufficient of the 0.02  $N$  acid to maintain the faint red color. To insure greatest possible accuracy, check strength of standard acid used by titration against pure morphine. 1 ml of 0.02  $N$   $H_2SO_4$  = 0.00570 g of  $C_{17}H_{19}O_3N$ .

**NOTE:** In the various operations involving fixation and subsequent liberation of morphine by means of fixed alkali and  $NH_4Cl$ , the most careful attention should be paid to the manner of adding the reagents, since any undue excess of either might nullify the entire procedure. Any large excess of  $NaOH$  would naturally require for its reduction a correspondingly large quantity of  $NH_4Cl$ , the latter in turn yielding its equivalent of hydroxide, relatively large quantities of which, thru interaction with  $NaCl$ , tend to inhibit any permanent liberation of alkaloid and thus prevent complete extraction. Furthermore,  $NH_4Cl$  in large quantity operates retentively on the morphine in soln, due in part possibly to the formation of an alkaloidal hydrochloride.

## ACETANILID AND SODIUM SALICYLATE (6)—OFFICIAL

## 39.16

## PREPARATION OF SOLUTION

Weigh quantity of powdered sample equal to, or multiple of, an average unit dose (ca 0.2 g of acetanilid); transfer to separator containing 10 ml of  $\text{H}_2\text{O}$ ; and for every unit dose add 0.1 g of solid  $\text{NaHCO}_3$ . In the examination of alcoholic preparations, distil the alcohol from a measured volume on steam bath, transfer to separator with minimum quantity of  $\text{H}_2\text{O}$ , and add 0.5–1.0 g of solid  $\text{NaHCO}_3$ .

## 39.17

## DETERMINATION

(a) *Acetanilid*.—Extract the alkaline soln, 39.16, with three 50 ml portions of  $\text{CHCl}_3$ ; wash each portion with 5 ml of  $\text{H}_2\text{O}$  in second separator and collect the solvent, without previous drying, in 200 ml Erlenmeyer flask. Reserve aqueous soln for determination of Na salicylate, (b). Distil the  $\text{CHCl}_3$  very gently to ca 5 ml, add 10 ml of  $\text{H}_2\text{SO}_4$  (1+9), and completely hydrolyze on steam bath. Proceed as directed under 39.7(b), beginning "Add 10 ml of  $\text{HCl}$ ."

(b) *Sodium salicylate*.—Acidify the aqueous soln of Na salicylate, (a), with a few drops of  $\text{HCl}$  and extract 3 to 5 times with 25 ml portions of  $\text{CHCl}_3$  to remove the salicylic acid. Treat each portion in second separator with 20 ml of  $\text{H}_2\text{O}$  containing 1 g of anhydrous  $\text{Na}_2\text{CO}_3$  for every 0.1 g of salicylic acid. Shake vigorously, and after clearing wash each portion again in third separator with 5 ml of  $\text{H}_2\text{O}$ . Add washings to main aqueous alkaline soln of Na salicylate. Dilute to known volume; transfer an aliquot, representing ca 0.1 g of salicylic acid, to 200 ml Erlenmeyer flask; dilute to 60–75 ml; heat nearly to boiling; add slowly 50–80 ml of ca 0.1 *N*  $\text{I}$ , sufficient to insure an excess during digestion; and digest for an hour on steam bath. Remove the free  $\text{I}$  with a few drops of  $\text{Na}_2\text{S}_2\text{O}_3$  soln and decant clear liquid thru weighed Gooch crucible, retaining most of precipitate, tetraiodophenylenequinone  $(\text{C}_6\text{H}_2\text{I}_2\text{O})_2$ , in the flask. To the latter add 50 ml of boiling  $\text{H}_2\text{O}$ , digest 10 min. on steam bath, filter, and gradually wash all precipitate into Gooch crucible, using for this purpose and final washings ca 200 ml of hot  $\text{H}_2\text{O}$ . Dry precipitate to constant weight in air bath at  $100^\circ$ .  $(\text{C}_6\text{H}_2\text{I}_2\text{O})_2 \times 0.4654 = \text{NaC}_7\text{H}_5\text{O}_3$ .

Should mixture contain antipyrine or caffeine or both, these substances will appear with the acetanilid in first  $\text{CHCl}_3$  extract and may be determined as directed in remarks following 39.47(b). Should the acetanilid be replaced by acetophenetidin in the mixture, the general procedure would not be materially altered, the acetophenetidin being weighed directly after recovery from its washed  $\text{CHCl}_3$  soln as separated from the Na salicylate. If, instead of Na salicylate, the mixture contains the free acid or its  $\text{NH}_4$  salt, add a larger quantity of  $\text{NaHCO}_3$  prior to extraction with  $\text{CHCl}_3$  to insure the fixation of salicylic acid.

In the analysis of a mixture of caffeine, acetanilid, Na salicylate, and codeine, the following procedure is recommended: (1) Extraction of caffeine, acetanilid, and salicylic acid from the acidified soln; (2) washing the  $\text{CHCl}_3$  soln with aqueous  $\text{Na}_2\text{CO}_3$  soln for recovery of the salicylic acid, preliminary to its treatment with  $\text{I}$  soln; (3) separation of caffeine and acetanilid as directed under 39.7(b); and (4) recovery of codeine from the soln of its sulfate after treatment with  $\text{NaHCO}_3$  and  $\text{CHCl}_3$ .

## ACETOPHENETIDIN (PHENACETIN) AND CAFFEINE (7)—OFFICIAL

## 39.18

## PREPARATION OF SOLUTION

In preparations containing acetophenetidin instead of acetanilid, but otherwise identical, make the gross separation of the caffeine-acetophenetidin mixture as directed under 39.6.

## 39.19

## DETERMINATION

(a) *Caffeine*.—Treat the  $\text{CHCl}_3$  extract, 39.18, with 10 ml of  $\text{H}_2\text{SO}_4$  (1+9) and digest on steam bath until liquid is reduced to ca 5 ml. Dilute with 10 ml of  $\text{H}_2\text{O}$  and continue digestion until volume is again reduced to 5 ml; again add 10 ml of  $\text{H}_2\text{O}$  and continue heating until residual liquid amounts to 5 ml. Repeat the diluting and evaporating until odor of acetic acid can no longer be detected in the vapors. If, during digestion, particles of acetophenetidin remain on sides of flask, rinse them into the soln with a few drops of  $\text{CHCl}_3$ .

Cool, transfer with  $\text{H}_2\text{O}$  to separator so that the final volume does not greatly exceed 20 ml, and proceed as directed under 39.7(a).

NOTE: Great care must also be given to the degree of evaporation. Should the aqueous-acid soln and suspension of caffeine-acetophenetidin be concentrated much beyond the limits indicated, more or less phenetidin sulfonate is likely to be formed, which later resists acetylation and conversion to acetophenetidin.

(b) *Acetophenetidin*.—Wash the filter used to dry the  $\text{CHCl}_3$  with 5 ml of  $\text{H}_2\text{O}$  receiving the washings in the separator containing the soln of phenetidin sulfate. Treat with successive small portions of solid  $\text{NaHCO}_3$  until, after complete neutralization of free acid, an excess of  $\text{NaHCO}_3$  remains. Add 50 ml of  $\text{CHCl}_3$ , and for every 0.1 g of acetophenetidin known or believed to have been present, add 5 drops of acetic anhydride. Shake vigorously, allow to clear, and withdraw the  $\text{CHCl}_3$  into second separator containing 5 ml of  $\text{H}_2\text{O}$ . Shake this mixture, and after clearing pass the solvent thru small, dry filter into 250 ml Erlenmeyer flask. Repeat extraction twice with 50 ml portions of  $\text{CHCl}_3$ , washing each portion with the 5 ml of  $\text{H}_2\text{O}$  in the second separator. Distil the combined  $\text{CHCl}_3$  extractions to ca 10 ml, transfer residual soln with sufficient fresh solvent to weighed 50 ml beaker or crystallizing dish, evaporate on steam bath to apparent dryness, and finally remove any considerable excess of acetic anhydride by repeated additions and evaporations of 1 ml of  $\text{CHCl}_3$  and a drop of alcohol. (Reformed acetophenetidin should finally appear as a whitish, crystalline mass with faint, acetous odor that disappears completely on standing some hours in open or over  $\text{CaO}$  in vacuum desiccator.) Weigh at intervals until final weight differs from preceding by not more than 0.0005 g.

## ACETOPHENETIDIN (PHENACETIN) AND SALOL (S)

## 39.20

*Acid Hydrolysis Method—Tentative*

(a) *Acetophenetidin*.—Weigh on a tared 5.5 cm filter a quantity of sample equal to, or multiple of, average weight of unit dose and wash with sufficient successive small portions of  $\text{CHCl}_3$  to extract completely all acetophenetidin and salol present in the mixture (ca 40 ml). Collect soln in weighed 100 ml beaker and evaporate on warm plate (50–60°) to apparent dryness, using air blast. Let stand 24 hours at room temp. to practically constant weight and weigh. By means of  $\text{CHCl}_3$  transfer crystalline residue to 50 ml lipped Erlenmeyer flask, evaporate solvent by means of air blast and gentle heat, add 10 ml of  $\text{H}_2\text{SO}_4$  (1+9), and evaporate on steam bath until volume is reduced one-half. Add 10 ml of  $\text{H}_2\text{O}$  and continue digestion as before. Add a second 10 ml of  $\text{H}_2\text{O}$  and evaporate to 5 ml. Transfer residue with ca 20 ml of  $\text{H}_2\text{O}$  to small separator and extract the salol with 15, 10, and 5 ml of  $\text{CHCl}_3$ , washing each extract with 5 ml of  $\text{H}_2\text{O}$  in second separator. Add wash  $\text{H}_2\text{O}$  in second separator to soln of phenetidin sulfate in first separator and proceed as directed under 39.19(b), beginning, "Treat with successive small portions of solid  $\text{NaHCO}_3$ ."

(b) *Salol*.—Subtract weight of acetophenetidin from combined weight of the two ingredients to obtain weight of salol.

## 39.21

*Alkaline Hydrolysis Method—Tentative*

(a) *Acetophenetidin*.—On a small, tared filter or in a small beaker weigh a quantity of the sample containing not more than 0.08 g of salol; exhaust with  $\text{CHCl}_3$ , as directed under 39.20(a); collect solvent in small lipped Erlenmeyer flask; and evaporate the  $\text{CHCl}_3$  by means of air blast without heat. Add 10 ml of 2.5%  $\text{NaOH}$  soln and heat 5 min. on steam bath. Cool quickly to room temp. in running  $\text{H}_2\text{O}$ . Transfer liquid to separator with minimum quantity of  $\text{H}_2\text{O}$  and rinse flask with first 20 ml portion of  $\text{CHCl}_3$  to be used in the following extraction. Extract the alkaline soln with three 20 ml portions of  $\text{CHCl}_3$ ; wash each portion in second separator with 5 ml of  $\text{H}_2\text{O}$ , and pass the  $\text{CHCl}_3$  soln thru small, dry filter into 200 ml Erlenmeyer flask. Reserve combined alkaline soln and washings for determination of salol (b). Distil combined  $\text{CHCl}_3$  extracts to ca 5 ml. Transfer by means of a little  $\text{CHCl}_3$  to small, weighed beaker or crystallizing dish, evaporate on steam bath with aid of air blast, cool, and weigh residual acetophenetidin at intervals until weight becomes constant.

(b) *Salol*.—Place the reserved combined alkaline soln and washings (a) in 500 ml I flask, dilute with  $\text{H}_2\text{O}$  to ca 200 ml, run in from buret an excess (ca 50 ml) of 0.1 *N* bromide-bromate, 39.28(c), add 10 ml of  $\text{HCl}$ , and shake 1 min., then at intervals for 30 min. Add 10 ml of 15%  $\text{KI}$  soln and shake at intervals for 15 min. Titrate the free I with standard  $\text{Na}_2\text{S}_2\text{O}_3$  soln previously standardized against the 0.1 *N* bromide-bromate. 1 ml of 0.1 *N* bromide-bromate = 0.00178 g of salol.

## ACETYSALICYLIC ACID (9)

## 39.22

*Melting Point—Official*

If excipients are present, treat 0.2–0.3 g with small portions of  $\text{CHCl}_3$  and filter into beaker or evaporating dish. Evaporate bulk of  $\text{CHCl}_3$  on steam bath and complete by spontaneous evaporation until thoroly dry. Determine melting point of crystalline residue by U.S.P. method.

## FREE SALICYLIC ACID

## 39.23

*Qualitative Test—Official*

Shake 0.5 g sample in small Erlenmeyer flask with ca 10 ml of  $\text{CHCl}_3$  and filter. Evaporate, treat residue with 10 ml of cold  $\text{H}_2\text{O}$ , and filter. Add 1 drop of 10%  $\text{FeCl}_3$  soln. Only a very faint violet color should result.

*Quantitative Method—Official*

## 39.24

## REAGENTS

(a) *Standard salicylic acid soln*.—Dissolve 0.01 g of salicylic acid in 100 ml of alcohol. Use only a freshly prepared soln.

(b) *Ferric ammonium sulfate soln*.—Add 1 ml of normal  $\text{HCl}$  to 2 ml of 8%  $\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$  soln and dilute with  $\text{H}_2\text{O}$  to 100 ml.

## 39.25

## PREPARATION OF SAMPLE.—See 39.1.

## 39.26

## DETERMINATION

In each of two colorimeter tubes mix 48 ml of  $\text{H}_2\text{O}$  and 1 ml of the freshly prepared  $\text{FeNH}_4(\text{SO}_4)_2$  soln. Shake 2.5 g of the powdered sample with exactly 25 ml of alcohol and filter if necessary. Immediately add 1 ml of the filtrate to one of the colorimeter tubes and 1 ml of the standard salicylic acid soln to the other, and mix. Immediately and rapidly make color comparisons and calculate the free salicylic

acid on the basis of the acetylsalicylic acid present. If color is too intense for satisfactory comparison, repeat entire determination, using smaller weight of the powdered sample.

#### TOTAL SALICYLATES

##### 39.27

##### *Iodine Method (9)—Official*

Weigh sufficient sample to yield 0.1–0.2 g of acetylsalicylic acid into beaker, add 20 ml of  $H_2O$  and 1 g of  $Na_2CO_3$ , and heat on steam bath 15 min. Filter, if necessary, to remove talc. Dilute to 60–75 ml, heat nearly to boiling, add slowly an excess (50–80 ml) of ca 0.1 *N* I, and proceed as directed under 39.17(b). Multiply weight of precipitate by 0.4015 to obtain total salicylic acid and deduct the free salicylic acid, 39.26. Multiply remainder by 1.304 to obtain weight of acetylsalicylic acid.

##### *Bromine Method—Official*

##### 39.28

##### REAGENTS

- (a) *Sodium hydroxide soln.*—Dissolve 2 g of NaOH in  $H_2O$  and dilute to 100 ml.
- (b) *Potassium iodide soln.*—Dissolve 20 g of KI in  $H_2O$  and dilute to 100 ml.
- (c) *Standard bromide-bromate soln (0.1 N bromine soln).*—Dissolve 3 g of  $KBrO_3$  and 12 g of KBr in  $H_2O$  and dilute to 1 liter. Standardize 30 ml of the KBr- $KBrO_3$  soln by transferring to I flask and adding 25 ml of  $H_2O$ , 5 ml of the KI soln, and 5 ml of HCl. Shake thoroly. Titrate with 0.1 *N*  $Na_2S_2O_3$ , using starch indicator, 6.3(e).

##### 39.29

##### DETERMINATION

Saponify 0.5 g sample with 10 ml of the NaOH soln by heating 15 min. on steam bath. Dilute with  $H_2O$  in volumetric flask to 500 ml. Transfer aliquot of this soln, representing not less than 0.04 g nor more than 0.05 g of acetylsalicylic acid, to 500 ml I flask; add 30 ml of the standard bromide-bromate soln and 5 ml of HCl and immediately insert stopper. Shake repeatedly 30 min. and allow to stand 15 min. Remove stopper just sufficiently to introduce quickly 5 ml of the KI soln, taking care that no Br vapors escape, and immediately stopper flask. Shake thoroly, remove stopper, and rinse it and neck of flask with a little  $H_2O$  so that washings flow into flask. Titrate with 0.1 *N*  $Na_2S_2O_3$ , using starch indicator, 6.3(e). 1 ml of 0.1 *N* bromide-bromate = 0.00230 g of salicylic acid, or 0.00300 g of acetylsalicylic acid.

##### *Double Titration Method for Acetylsalicylic Acid (10)—Official*

##### 39.30

##### PREPARATION OF SOLUTION

- (a) *Dry extraction method (applicable in all cases).*—Treat weighed quantity of sample containing not less than 0.3 g of acetylsalicylic acid with small portions of  $CHCl_3$ , filter into beaker, and wash residue with  $CHCl_3$  until completely extracted. Evaporate bulk of the  $CHCl_3$  on steam bath, finishing with aid of electric fan without heat.
- (b) *Wet extraction method (applicable in absence of acids and alkalies, or alkaline earth carbonates).*—Transfer the accurately weighed sample to small separator containing ca 20 ml of  $H_2O$ . Shake out repeatedly with  $CHCl_3$ , using successively 30, 25, 20, 15, 10, and 5 ml portions, and test for completeness of extraction by evaporating a portion of final extraction on watch-glass. Filter combined  $CHCl_3$  portions thru cotton and wash funnel and cotton with  $CHCl_3$ . Evaporate bulk of the  $CHCl_3$  on steam bath, finishing with aid of electric fan without heat.
- (c) *Acetylsalicylic acid and uncoated tablets containing no excipient.*—Dissolve sample directly in 10 ml of neutral alcohol.

## 39.31

## DETERMINATION

Dissolve the dry  $\text{CHCl}_3$  extract in 10 ml of neutral alcohol and titrate immediately and rapidly with 0.1 *N* alkali soln, using phenolphthalein indicator. Use the first persistent pink color as the end point, since any slight excess of alkali has a tendency to hydrolyze the ester quickly. Add a volume of the 0.1 *N* alkali equal to that used in the first titration and then add 5 ml more. Heat on steam bath 15 min. Titrate back with 0.1 *N* acid. If product is pure, the total quantity of alkali consumed will be twice that of first titration. Each ml of 0.1 *N* alkali consumed in the 2 titrations = 0.0090 g of acetylsalicylic acid.

## 39.32

## COMBINED ACETIC ACID IN ACETYLSALICYLIC ACID (11)—OFFICIAL

If excipients are present, weigh accurately 2 g of the powdered material and transfer to separator, using ca 25 ml of  $\text{H}_2\text{O}$ . Extract completely with  $\text{CHCl}_3$ , testing last extraction by evaporating small quantity of the  $\text{CHCl}_3$  to dryness. (Usually 6 extractions with 30, 25, 20, 10, 10, and 5 ml portions of  $\text{CHCl}_3$  are sufficient.) Filter the  $\text{CHCl}_3$  fractions thru pledget of cotton into a beaker. Wash original beaker, funnel, and cotton with  $\text{CHCl}_3$  and add these washings to  $\text{CHCl}_3$  soln in beaker. Evaporate the  $\text{CHCl}_3$  on steam bath; dry residue at  $80^\circ$  for 15 min.

Treat the  $\text{CHCl}_3$  extract, or if no excipients are present, 2 g of the powdered material, in 150 ml beaker with 30 ml of *N* NaOH and evaporate on steam bath nearly to dryness. Transfer to separator, using 10 ml of  $\text{H}_2\text{O}$ , 20 ml of 10%  $\text{H}_2\text{SO}_4$ , and finally two 5 ml portions of  $\text{H}_2\text{O}$ . Extract with successive portions of  $\text{CHCl}_3$ , using first fraction of 50 ml to rinse beaker in which saponification was carried on. Continue the extractions with  $\text{CHCl}_3$  until all salicylic acid is removed (ca 6 extractions). During these extractions keep stopper in separator to guard against loss of acetic acid by evaporation. Collect the  $\text{CHCl}_3$  fractions in second separator, wash with 25 ml of  $\text{H}_2\text{O}$ , and wash this  $\text{H}_2\text{O}$  once with 5 ml of  $\text{CHCl}_3$ . Discard the  $\text{CHCl}_3$  extractions and return wash  $\text{H}_2\text{O}$  to acid  $\text{H}_2\text{O}$  in first separator. Transfer the acid  $\text{H}_2\text{O}$  containing acetic acid and  $\text{H}_2\text{SO}_4$  to 200 ml volumetric flask, wash separators thoroly with  $\text{H}_2\text{O}$ , add to flask, dilute to volume, and mix thoroly. Pipet two 50 ml portions, using same pipet and draining same length of time. Place one portion in receptacle suitable for titration and the other in large Pt dish. Titrate first portion at once with 0.5 *N* alkali, using phenolphthalein indicator. Evaporate portion in Pt dish on steam bath to dryness, take up in 10 ml of  $\text{H}_2\text{O}$ , and again evaporate, repeating this process twice more. (During evaporation guard against contact with  $\text{NH}_3$  vapors.) Take up residue in  $\text{H}_2\text{O}$  and titrate with 0.5 *N* alkali, using phenolphthalein indicator. Subtract second titration reading from first and calculate percentage of acetic acid on a 0.5 g sample. 1 ml of 0.5 *N* alkali = 0.0300 g of acetic acid.

## 39.33

## ACETYLSALICYLIC ACID IN MIXTURES CONTAINING ACETOPHENETIDIN AND CAFFEINE (12)—OFFICIAL

Ascertain average weight of a number of tablets and reduce to fine powder.

Weigh ca 0.2 g of the powder, transfer to separator with ca 25 ml of  $\text{H}_2\text{O}$ , and extract carefully with repeated portions of  $\text{CHCl}_3$ . Test final extraction by evaporating small portion on steam bath to dryness; ca 6 extractions are generally required, and these can be made with 30, 25, 20, 10, 10, and 5 ml portions of  $\text{CHCl}_3$ . Collect the  $\text{CHCl}_3$  fractions in separator and draw off into 200 ml Erlenmeyer flask, placing pledget of cotton in stem of separator to filter the  $\text{CHCl}_3$ . Wash separator twice with 5 ml portions of  $\text{CHCl}_3$ , passing this thru the cotton and leaving any  $\text{H}_2\text{O}$  that may have separated in the separator. Add  $\text{CHCl}_3$  washings to flask and evaporate the  $\text{CHCl}_3$  on steam bath to volume of ca 2 ml. Add 10 ml



of  $\text{H}_2\text{SO}_4$  (1+9), connect with reflux condenser, and digest 30 min., partially immersing flask in boiling water bath. Cool, and transfer to separator, rinsing condenser with  $\text{CHCl}_3$ , and using minimum quantity of  $\text{H}_2\text{O}$  to effect transfer, so that final volume does not greatly exceed 20 ml. Extract the caffeine and salicylic acid with 6 portions of  $\text{CHCl}_3$ , using 30, 25, 20, 15, 10, and 10 ml. Collect these fractions in separator, add 20 ml of  $\text{H}_2\text{O}$  and 1 g of  $\text{Na}_2\text{CO}_3$ , and shake thoroly. Drain the  $\text{CHCl}_3$  into another separator and wash twice more with 15 and 10 ml of  $\text{H}_2\text{O}$ . Reject the  $\text{CHCl}_3$  and combine the  $\text{Na}_2\text{CO}_3$  soln and wash waters in 200 ml Erlenmeyer flask. Heat on steam bath to expel traces of  $\text{CHCl}_3$ , dilute to 100 ml with  $\text{H}_2\text{O}$ , then add slowly 25–40 ml of strong I soln (ca 0.2 *N*), sufficient to insure excess during digestion, and digest 1 hour on steam bath. Remove free I with a few drops of  $\text{Na}_2\text{S}_2\text{O}_3$  soln. Decant clear soln thru weighed Gooch, retaining most of precipitate in flask. To latter add 50 ml of boiling  $\text{H}_2\text{O}$ , digest 10 min. on steam bath, filter, and wash gradually all the precipitate into the Gooch, using altogether ca 200 ml of hot  $\text{H}_2\text{O}$ . Dry to constant weight in air bath at  $100^\circ$  and weigh precipitate of tetraiodophenylenequinone ( $\text{C}_6\text{H}_2\text{I}_4\text{O}$ )<sub>2</sub>. Weight of precipitate  $\times 0.4016$  = total salicylic acid present. If free salicylic acid is present, deduct from total; difference  $\times 1.304$  = weight of acetylsalicylic acid.

#### ACETOPHENETIDIN, ACETYSALICYLIC ACID, AND CAFFEINE (15)—TENTATIVE

##### 39.34

##### REAGENTS

(a) *Sulfuric acid soln.*—2%. Pour 6.0 ml of  $\text{H}_2\text{SO}_4$  into 500 ml of  $\text{H}_2\text{O}$ .

(b) *Sodium bicarbonate soln.*—Freshly prepared. Add 3 g of  $\text{NaHCO}_3$  to 45 ml of  $\text{H}_2\text{O}$  previously cooled to  $15^\circ$  or lower. Stir until dissolved and add 2–3 drops of 10%  $\text{HCl}$ .

##### 39.35

##### DETERMINATION

(a) *Acetylsalicylic acid.*—Make determination as soon as possible to prevent any hydrolysis in the  $\text{NaHCO}_3$  soln.

Weigh sufficient powdered sample to represent at least 0.04 g of caffeine, transfer to separator containing ca 10 ml of  $\text{H}_2\text{O}$  cooled to  $15^\circ$  or lower, and shake thoroly. Add 15 ml of the cooled  $\text{NaHCO}_3$  soln slowly to prevent mechanical loss due to effervescence and immediately extract with successive portions of  $\text{CHCl}_3$ . Wash each portion of  $\text{CHCl}_3$  thru second separator containing 2 ml of the cold  $\text{NaHCO}_3$  soln and filter thru cotton moistened with  $\text{CHCl}_3$ . (Extraction is complete when a final shakeout evaporated to dryness leaves a negligible residuc. Usually 5 extractions with ca 30 ml portions of  $\text{CHCl}_3$  are sufficient.) Set aside combined  $\text{CHCl}_3$  extracts containing caffeine and acetophenetidin for later treatment. Transfer wash  $\text{H}_2\text{O}$  in second separator to soln in first separator, rinsing several times with small portions of  $\text{H}_2\text{O}$ . Acidify combined  $\text{NaHCO}_3$  solns with  $\text{HCl}$  (1+1) and extract the acetylsalicylic acid by shaking with successive portions of  $\text{CHCl}_3$ , filtering each portion thru funnel containing pledget of cotton moistened with  $\text{CHCl}_3$  (usually 5 extractions are sufficient). Evaporate combined  $\text{CHCl}_3$  extracts on steam bath with aid of fan or gentle air blast until volume is ca 10 ml. Transfer to suitable small tared container with aid of  $\text{CHCl}_3$  and evaporate to dryness by means of fan or gentle air blast without heat. Dry in desiccator overnight and weigh as acetylsalicylic acid. Extracted acetylsalicylic acid may be checked by Br method or by double titration method (39.29 or 39.31).

(b) *Acetophenetidin and caffeine.*—Evaporate the  $\text{CHCl}_3$  soln containing the acetophenetidin and caffeine on steam bath and transfer, when volume reaches 5–10 ml, to 100 ml beaker by means of small portions of  $\text{CHCl}_3$ . Evaporate again

to volume of ca 5 ml and add 10 ml of 2%  $\text{H}_2\text{SO}_4$ . Introduce a stirring rod and heat mixture on bath until all the  $\text{CHCl}_3$  has evaporated, stirring occasionally. Cool to room temp. and decant thru tared Gooch crucible previously dried to constant weight at  $100^\circ$  (no suction required). Collect filtrate in 150 ml beaker, retaining as much of the acetophenetidin as possible in beaker. Rinse sides of beaker containing the acetophenetidin with 5–10 ml of  $\text{CHCl}_3$ , add 10 ml of 2%  $\text{H}_2\text{SO}_4$ , and heat on bath as before until all  $\text{CHCl}_3$  has evaporated. Cool, and decant thru same crucible as before. Repeat process with another 10 ml portion of the  $\text{H}_2\text{SO}_4$ , and finally wash the acetophenetidin quantitatively into crucible with  $\text{H}_2\text{O}$ . Wash beaker and crucible with  $\text{H}_2\text{O}$  until filtrate measures ca 75 ml. Dry crucible at  $100^\circ$  and weigh the acetophenetidin.

To filtrate containing the caffeine and the small amount of acetophenetidin that went into soln (ca 0.075 g), add 5 ml of  $\text{H}_2\text{SO}_4$  (1+9) and evaporate on steam bath to volume of ca 10 ml. Transfer by means of small portions of  $\text{H}_2\text{O}$  to 50 ml Erlenmeyer flask previously marked for volumes of 5 and 10 ml. Proceed as directed in 39.19(a), bearing in mind that hydrolysis must be continued until no odor of acetic acid is present. Hydrolysis is hastened somewhat if flask is allowed to hang in the steam from wire wrapped around its neck so that mouth of flask is about level with surface of the bath (ca 3 evaporations are usually sufficient). Add weight of acetophenetidin obtained to weight of acetophenetidin collected in the Gooch crucible to obtain total acetophenetidin content of the sample.

**ACETYSALICYLIC ACID, ACETOPHENETIDIN, AND SALOL (14)—OFFICIAL,  
FIRST ACTION**

**39.36**

**REAGENTS**

- (a) *Sodium bicarbonate soln.*—Prepare fresh as directed in 39.34(b).
- (b) *Standard bromide-bromate soln* (0.1 *N* bromine soln).—See 39.28(c).
- (c) *Standard  $\text{Na}_2\text{S}_2\text{O}_3$  soln.*—0.1 *N*.
- (d) *Sodium hydroxide soln.*—2.5% w/v NaOH.

**39.37**

**DETERMINATIONS**

(a) *Acetylsalicylic acid.*—Make determination as soon as possible to prevent any hydrolysis in the  $\text{NaHCO}_3$  soln.

Weigh 0.5–1.0 g of powdered sample and proceed as directed in 39.35(a).

(b) *Acetophenetidin and salol.*—Evaporate combined  $\text{CHCl}_3$  solns containing the acetophenetidin and salol [corresponding to acetophenetidin and caffeine, 39.35(b)] to dryness by means of gentle air blast or fan without heat. Dissolve residue in a few ml of ether and again evaporate to dryness. Treat residue as directed in 39.21(a), beginning "Add 10 ml of 2.5% NaOH soln." For salol, change procedure as follows: Transfer the alkaline salol soln, freed from acetophenetidin, to volumetric flask and make to volume with  $\text{H}_2\text{O}$ . Take an aliquot of this soln, containing quantity of salol not exceeding 0.08 g, for bromination.

**ACETYSALICYLIC ACID AND PHENOLPHTHALEIN IN TABLETS (15)—OFFICIAL,  
FIRST ACTION**

**39.38**

**PREPARATION OF SAMPLE.**—See 39.1

**39.39**

**DETERMINATIONS**

Weigh sufficient powdered material to contain 0.05–0.1 g of phenolphthalein. Extract the dry powder repeatedly with 20 ml portions of ether and filter into separator. Test for complete extraction (5–8 extractions required).

(a) *Acetylsalicylic acid*.—Shake the ethereal soln for at least 1 min. each time with two 20 ml portions of 4%  $\text{NaHCO}_3$  soln (temp.  $20^\circ$  or less). Transfer soln to second separator. Wash the ether with two 10 ml portions of  $\text{H}_2\text{O}$  and add to bicarbonate soln. Extract bicarbonate soln with 20 ml of ether. Draw off lower aqueous layer into 100 ml volumetric flask. Wash ether with small portions of  $\text{H}_2\text{O}$ , rinse into flask, and dilute to mark. Add wash ether to bulk of solvent in original separator. Reserve ethereal soln for determination of phenolphthalein.

Transfer an aliquot of the bicarbonate soln containing not less than 0.3 g of acetylsalicylic acid to separator. (Acid must be isolated from the bicarbonate soln as rapidly as possible to prevent hydrolysis.) Acidify with 10%  $\text{HCl}$  and extract the liberated acetylsalicylic acid with 30, 20, 20, 10, and 10 ml portions of  $\text{CHCl}_3$ -ether solvent (3+2). Wash each extract with 2 ml of  $\text{H}_2\text{O}$  in second separator and filter thru pledget of cotton, moistened with solvent, into counterpoised tared beaker. Test for complete extraction. Evaporate solvent to 10–15 ml on water bath and complete evaporation without aid of heat. Dry residue to constant weight at room temp. Weight may be checked by double titration method, 39.31.

(b) *Phenolphthalein*.—Extract original ethereal soln with 20 ml portions of 3%  $\text{NaOH}$  soln until all phenolphthalein has been removed as indicated by color. Transfer these alkaline extracts to second separator, acidify with 10%  $\text{HCl}$ , and extract with  $\text{CHCl}_3$ -ether solvent (3+2). Wash each portion of extract in third separator with 2 ml of  $\text{H}_2\text{O}$  to which has been added 1 or 2 drops of 10%  $\text{HCl}$ . Filter extracts into counterpoised tared beaker, using in stem of funnel pledget of cotton moistened with the  $\text{CHCl}_3$ -ether mixture. Evaporate on water bath and dry to constant weight at  $120^\circ$ . Weight may be checked by tetraiodo method, 39.17(b).

#### SALICYLIC ACID IN PRESENCE OF OTHER PHENOLS (16)—OFFICIAL

##### 39.40

##### PREPARATION OF SAMPLE

(a) *Powders*.—Weigh into volumetric flask such a quantity of material that an aliquot of 25–50 ml will contain ca 0.13 g of phenol. If acid, make alkaline with 4%  $\text{NaOH}$ , adding 25 ml in excess, fill to mark with  $\text{H}_2\text{O}$ , and shake well.

(b) *Liquids*.—Proceed as directed under 39.41.

##### 39.41

##### DETERMINATION

Transfer to a separator sufficient quantity of soln to represent ca 0.13 g of phenol. Acidify with 10%  $\text{H}_2\text{SO}_4$  and extract with ether, using 20, 15, 15, and 10 ml portions, until extraction is completed. Combine ether extracts in second separator. Shake with saturated  $\text{NaHCO}_3$  soln, using 15, 15, and 10 ml portions, and finally shake with 15 ml of  $\text{H}_2\text{O}$ . Combine the  $\text{NaHCO}_3$  solns and washings and extract combined  $\text{NaHCO}_3$  extracts with 15 ml of ether. Add latter to main bulk of ether and reserve for phenol determination. Acidify the  $\text{NaHCO}_3$  soln with  $\text{HCl}$ . Extract with  $\text{CHCl}_3$ -ether (2+1), using 30, 25, 20, and 10 ml until salicylic acid is completely removed. Filter extracts into beaker thru cotton previously saturated with  $\text{CHCl}_3$ . Evaporate to 5 ml on covered steam bath with aid of electric fan, allowing last 5 ml to evaporate spontaneously. Dissolve residue in 10 ml of neutral alcohol and titrate with 0.1  $N$   $\text{NaOH}$ , using phenolphthalein indicator. 1 ml of 0.1  $N$   $\text{NaOH}$  = 0.01381 g of salicylic acid,  $\text{C}_6\text{H}_4\text{OHCOOH}$ .

#### AMINOPYRINE (PYRAMIDON)

##### 39.42

##### Qualitative Tests(17)—Official

(a) Dissolve 0.01 g of sample in 2 ml of  $\text{H}_2\text{O}$  and add a few drops of yellow  $\text{HNO}_3$  (containing  $\text{HNO}_2$ ). Purplish blue colored soln is produced.

(b) Dissolve 0.01 g of sample in 2 ml of  $H_2O$  and add 1 ml of 10%  $FeCl_3$  soln. Purple to violet color develops, but it becomes red on addition of  $H_2SO_4$  (1+9).

(c) Dissolve 0.1 g of sample in 2 ml of  $H_2O$  and add a few drops of 5%  $AgNO_3$  soln. After few seconds a purple to violet color is produced and on standing a deposit of metallic Ag results (useful for detecting aminopyrine in antipyrine).

(d) Dissolve 0.1–0.2 g of sample in 2 ml of  $H_2O$ , add 1 or 2 drops of 0.2%  $NaNO_2$  soln and a few drops of  $H_2SO_4$  (1+9), and shake a few seconds. A purplish blue color develops, then gradually disappears, leaving a colorless soln. Avoid excess of  $NaNO_2$  as it destroys the color (useful for detecting antipyrine in presence of aminopyrine). On addition of a few more drops of the  $NaNO_2$  soln and the dilute  $H_2SO_4$  a yellowish green colored soln remains after the disappearance of the purple coloration if antipyrine is present.

**39.43***Quantitative Method (18)—Official*

Place 1 g of powdered sample in 100 ml volumetric flask, add 60 ml of normal  $H_2SO_4$ , and shake several minutes to insure complete soln of the aminopyrine. Make up to mark with normal  $H_2SO_4$ . Filter, if not clear, thru dry filter, rejecting first part of filtrate. Pipet a 20 ml aliquot of the soln, or filtrate, into separator; make distinctly alkaline with either  $NH_4OH$  or 5%  $NaOH$ ; and shake out with 20, 15, 10, 10, and 5 ml portions of  $CHCl_3$ . Combine the  $CHCl_3$  extracts in second separator and wash with 2 ml of  $H_2O$ . Filter the  $CHCl_3$  soln into weighed beaker thru pledget of cotton saturated with  $CHCl_3$ . Extract the wash  $H_2O$  with 5 ml of  $CHCl_3$  and add this to combined  $CHCl_3$  extracts. Evaporate combined  $CHCl_3$  extracts just to dryness on water bath with aid of electric fan and dry residue in oven at temp. of boiling  $H_2O$  for 10 min. Cool in desiccator, and weigh as aminopyrine. Identify aminopyrine by means of its melting point and qualitative tests.

**39.44****AMINOPYRINE, ACETOPHENETIDIN, AND CAFFEINE (19)—  
OFFICIAL, FIRST ACTION**

(a) *Aminopyrine*.—Transfer 2 g of powdered mixture to separator, add 15 ml of 10% (w/v)  $H_2SO_4$  and 50 ml of  $CHCl_3$  and shake well. Draw off the  $CHCl_3$  into second separator and wash with 15 ml of 10%  $H_2SO_4$ . Filter  $CHCl_3$  into flask. Extract mixture in first separator with 5 more portions of 25 ml each of  $CHCl_3$ , washing each portion successively thru the diluted  $H_2SO_4$  as before, filtering, and collecting the  $CHCl_3$  in the flask. Test for complete extraction. Reserve this soln for determination of acetophenetidin and caffeine. Add acid washing in second separator to first separator. Render mixture alkaline with 10%  $NH_4OH$ , and remove aminopyrine by successive extractions with 25 ml portions of  $CHCl_3$ . Wash each  $CHCl_3$  extract in second separator with 5 ml of  $H_2O$  containing a few drops of  $NH_4OH$  and filter solvent thru cotton into tared beaker. Evaporate solvent. Add a few ml of anhydrous ether and again evaporate. Dry residue at  $80^\circ$  and weigh as aminopyrine.

(b) *Acetophenetidin and Caffeine*.—Proceed as directed in 39.35(b).

Examine residues obtained qualitatively to establish their identity.

**39.45****AMINOPYRINE, ACETOPHENETIDIN, PHENOBARBITAL,  
AND CAFFEINE (20)—TENTATIVE**

(a) *Aminopyrine*.—Transfer 2.5 g of powdered mixture into separator, add 15 ml of 10% (w/v)  $H_2SO_4$  and 50 ml of  $CHCl_3$  and shake well. Draw off the  $CHCl_3$  into second separator and wash with 15 ml of 10%  $H_2SO_4$ . Filter the  $CHCl_3$  thru cotton into a flask. Extract mixture in first separator with 5 more portions of 25 ml

each of  $\text{CHCl}_3$ , washing each portion successively thru the diluted  $\text{H}_2\text{SO}_4$  as before, filtering and collecting the  $\text{CHCl}_3$  in the flask. Test for complete extraction. Reserve this soln for determination of acetophenetidin, caffeine, and phenobarbital. Add acid washing in second separator to first separator. Render mixture alkaline with  $\text{NH}_4\text{OH}$  (10%  $\text{NH}_3$ ) and remove the aminopyrine by successive extractions with 25 ml portions of  $\text{CHCl}_3$ . Wash each  $\text{CHCl}_3$  extract in second separator with 5 ml of  $\text{H}_2\text{O}$  containing a few drops of the  $\text{NH}_4\text{OH}$  and filter solvent thru cotton into tared beaker. Evaporate solvent. Add a few ml of anhydrous ether and again evaporate. Dry residue at  $80^\circ$  and weigh as aminopyrine.

(b) *Phenobarbital*.—Evaporate the  $\text{CHCl}_3$  soln of acetophenetidin, phenobarbital, and caffeine to ca 50 ml, transfer soln to separator, and shake five times with 25 ml portions of 0.1 *N*  $\text{NaOH}$ . Draw off aqueous alkaline solns and wash each successively with the same 5 ml portion of  $\text{CHCl}_3$ . Add  $\text{CHCl}_3$  washings to original  $\text{CHCl}_3$  soln of caffeine and acetophenetidin. Reserve this soln for further treatment. Acidify the aqueous alkaline soln with 10%  $\text{HCl}$  and shake with successive portions of 25 ml of  $\text{CHCl}_3$ -ether mixture (2+1). Wash each portion of the solvent successively with 10 ml of  $\text{H}_2\text{O}$  containing a few drops of  $\text{HCl}$ . Evaporate solvent, dry residue at  $80^\circ$ , and weigh as phenobarbital.

(c) *Acetophenetidin and Caffeine*.—Proceed as directed in 39.35(b).

#### ANTIPIRYNE AND CAFFEINE (21)—OFFICIAL

##### 39.46

##### PREPARATION OF SAMPLE

(a) Weigh quantity of finely powdered sample equal to, or multiple of, average unit dose; transfer to filter or beaker and extract with  $\text{CHCl}_3$ . Distil greater part of the  $\text{CHCl}_3$  and evaporate remainder on steam bath.

(b) With alcoholic preparations, remove alcohol from measured quantity by heating on steam bath. Extract residue with three 50 ml portions of  $\text{CHCl}_3$  in separator. Distil greater portion of  $\text{CHCl}_3$  and evaporate remainder on steam bath.

##### 39.47

##### DETERMINATION

(a) *Antipyrine*.—Transfer residue obtained, 39.46, which should weigh ca 0.25 g, to 125 ml separator by means of two 5 ml portions of alcohol-free  $\text{CHCl}_3$ , followed by 10 ml of  $\text{H}_2\text{O}$ . Add 1 g of  $\text{NaHCO}_3$  and 10–15 ml of 0.2 *N*  $\text{I}$  (or double quantity of 0.1 *N*  $\text{I}$ ), adding latter in small portions and shaking mixture vigorously after each addition. (The  $\text{I}$  should then be in excess of that required to convert all the antipyrine into mono-iodo derivative. If it is not, add a little more  $\text{I}$  and shake mixture again.) Remove free  $\text{I}$  with small crystal of  $\text{Na}_2\text{S}_2\text{O}_3$  and add 15 ml of washed  $\text{CHCl}_3$ , shaking vigorously 1 min. After clearing, draw off the  $\text{CHCl}_3$  into second separator; wash with 5 ml of  $\text{H}_2\text{O}$ , filter thru small, dry filter into weighed 50 ml beaker, and evaporate to apparent dryness on steam bath, using air blast. Repeat extraction with 2 (3, if 0.1 *N*  $\text{I}$  has been used) 25 ml portions of washed  $\text{CHCl}_3$ , washing, filtering, and evaporating each portion as directed previously. Recover any crystalline product separating about tip of delivery tube, funnel, and edge of filter by judicious washing with  $\text{CHCl}_3$ . Dry nearly colorless, crystalline residue of caffeine and iodoantipyrine 30 min. at  $100^\circ$ , cool, and weigh. Designate this weight as "A."

The use of alcohol-free  $\text{CHCl}_3$  in connection with the iodination of antipyrine is necessary in order to preclude the formation of  $\text{CHI}_3$ , presence of which in composite residue A would vitiate result.

Dissolve composite residue in 5 ml of acetic acid, add 10 ml of saturated  $\text{SO}_2$  soln, and wash with hot  $\text{H}_2\text{O}$  into 400–500 ml beaker until final volume is ca 200 ml. Add sufficient  $\text{AgNO}_3$  soln to precipitate all the I (ca 0.3 g of  $\text{AgNO}_3$ ) and a few drops of  $\text{HNO}_3$ , heat nearly to boiling, and stir to agglomerate the  $\text{AgI}$ . Add 15 ml of  $\text{HNO}_3$ , cover beaker with watch-glass, and boil gently 5 min. Decant thru weighed Gooch crucible; wash precipitate once with a little alcohol, then with two 100 ml portions of boiling  $\text{H}_2\text{O}$ ; and finally transfer  $\text{AgI}$  to crucible. Wash several times with hot  $\text{H}_2\text{O}$  and again with alcohol to remove traces of organic matter, dry 30 min. at  $110^\circ$ , cool, and weigh. Weight of  $\text{AgI} \times 0.8014 = \text{weight of antipyrine}$ .

(b) *Caffeine*.—Multiply weight of  $\text{AgI}$  by 1.3374 and subtract product from weight "A," under (a).

In the analysis of a mixture containing caffeine, antipyrine, acetanilid, and Na salicylate, following steps are essential in effecting a separation: (1) Extraction of caffeine, acetanilid, and antipyrine from the aqueous, alkaline soln with  $\text{CHCl}_3$ ; (2) hydrolytic treatment with  $\text{H}_2\text{SO}_4$  of the three substances thus separated, preliminary to determination of caffeine and antipyrine as directed under (a).

#### BARBITURATES (22)—OFFICIAL

##### 39.48 (Applicable in absence of stearic acid)

Weigh 0.3–0.5 g of sample into separator, add 10 ml of  $\text{H}_2\text{O}$  and shake well. Add 5 ml of 0.5 N  $\text{NaOH}$  and shake again. Acidify to litmus paper with 10%  $\text{HCl}$ , dropwise, and add ca 1 ml in excess. Shake out repeatedly with  $\text{CHCl}_3$ , using 40, 30, 20, 20, and 10 ml portions of solvent. Test for complete extraction by shaking with an additional 10 ml of solvent and evaporating in separate beaker.

Combine solvent in second separator and wash with 2 ml of  $\text{H}_2\text{O}$  acidified with a drop of  $\text{HCl}$ . Filter solvent thru pledget of cotton into small weighed beaker. Evaporate on steam bath with aid of electric fan, heat 10 min. at  $80\text{--}90^\circ$ , cool in desiccator, and weigh. Add 2 or 3 ml of anhydrous ether and evaporate solvent. (Usually 2 treatments with 2 ml each of anhydrous ether are sufficient to remove last traces of  $\text{CHCl}_3$  and to produce a crystalline residue.) Dry at  $80\text{--}90^\circ$ , cool, and weigh. Repeat treatment with anhydrous ether and evaporation until weight becomes constant. Determine melting point to check purity of residue.

##### 39.49 *Alternative Method (23)—Official*

(Applicable in presence of stearic acid)

Dissolve residue obtained in 39.48 in 10 ml of alcohol, add 20 ml of saturated  $\text{Ba}(\text{OH})_2$  soln, and stir well. Filter into separator and wash residue and filter with two or three 10 ml portions of the  $\text{Ba}(\text{OH})_2$  soln. Acidify filtrate with 10%  $\text{HCl}$  and proceed as directed under 39.48, beginning "Shake out repeatedly with  $\text{CHCl}_3$ ."

##### 39.50

#### CAMPHOR (24)—OFFICIAL

(Not applicable to synthetic camphor)

Weigh accurately into 400 ml round-bottomed Pyrex flask sufficient quantity of powdered material to contain ca 2 g of camphor. Add 10 ml of benzene and 10 ml of  $\text{H}_2\text{O}$  and connect flask with apparatus for steam distillation. Use 8–12" bulb condenser, well cooled, outlet of which reaches to bottom of 200 ml flask. Distil with steam, collecting the benzene and ca 100 ml of aqueous distillate. Disconnect condenser and wash it slowly with 5 ml of alcohol from pipet in such a manner as entirely to wet inside of condenser. Wash condenser in same manner with 10 ml of

benzene. Add both washings to contents of receiver. Saturate distillate with NaCl, add sufficient  $\text{H}_2\text{SO}_4$  (1 + 9) to insure acidity, transfer to separator, shake, and separate the two layers. Rinse original receiver with 10 ml of benzene and use rinsings to re-extract the aqueous soln. Separate aqueous layer and extract it once more with 10 ml of benzene. Wash combined benzene extracts with 10 ml of saturated NaCl soln rendered distinctly alkaline with  $\text{Na}_2\text{CO}_3$ . Separate layers and extract aqueous layer with 10 ml of benzene. Discard aqueous solns, transfer benzene to 50 ml volumetric flask, and make up to mark with benzene. Shake soln and filter into 200 mm polariscope tube, using water-jacketed tube, if necessary, to maintain constant temp. of  $20^\circ$ . Make 10 readings, using  $\text{K}_2\text{Cr}_2\text{O}_7$  filter, and take average reading for calculating the camphor. Calculate quantity of camphor ( $Q$ ) contained in the 50 ml of benzene and, therefore, in sample taken, from average reading in circular degrees ( $a$ ) by following formula:  $Q = 0.6171a - 0.0022a^2$ .

The value of  $Q$  does not vary directly with length of tube. If longer or shorter tube than directed is used, correct value of  $a$  to 200 mm tube, and then make calculation by above formula.

### MONOBROMATED CAMPHOR IN TABLETS

#### *Method I (25)—Official*

#### 39.51

##### REAGENT

*Sodium amalgam.*—Cut ca 1 g of bright metallic Na into small pieces and dissolve in 100 g of warm Hg, contained in small porcelain mortar, by impaling pieces successively on point of file and holding them submerged in the Hg until the rather violent action is complete. Keep resulting amalgam in a tightly corked bottle.

#### 39.52

##### PREPARATION OF SAMPLE.—See 39.1

#### 39.53

##### DETERMINATION

Weigh portion of powdered sample corresponding to 0.1–0.2 g. of monobromated camphor, and transfer with 20 ml of alcohol and 10 ml of  $\text{H}_2\text{O}$  to small (100 ml) round-bottomed flask containing 15 g of the Na amalgam. Connect flask by means of rubber stopper with vertical condenser. Boil mixture gently over wire gauze at least 30 min. Cool slightly and wash out condenser tube with 5 ml of alcohol and 5 ml of  $\text{H}_2\text{O}$ , receiving washings in flask. Place flask on steam bath and heat for another hour, or until evolution of H has nearly or quite ceased. Toward latter part of this operation, to facilitate reduction, render liquid about neutral with a few drops of acetic acid. Transfer contents of flask to separator, withdrawing the Hg into second separator and washing it with at least two 50 ml portions of  $\text{H}_2\text{O}$ . Pass the several aqueous solns thru small filter, collecting clear filtrate in suitable beaker. Precipitate with 10%  $\text{AgNO}_3$  soln, add ca 5 ml of  $\text{HNO}_3$ , and filter, collecting the AgBr in weighed Gooch crucible. Wash with  $\text{H}_2\text{O}$  and alcohol, dry at  $100^\circ$ , and weigh. Weight of  $\text{AgBr} \times 1.23$  = weight of monobromated camphor. Run a control on the amalgam to determine whether any correction is necessary.

#### *Method II (26)—Official, Fist Action*

#### 39.54

##### PREPARATION OF SAMPLE.—See 39.1

#### 39.55

##### DETERMINATION

Weigh in small beaker a quantity of the powdered sample equivalent to ca 0.2 g of monobromated camphor, add 25 ml of alcohol, warm on steam bath, and filter

into flask (preferably ca 250 ml capacity and provided with a ground-in condenser), washing both beaker and filter with warm alcohol. Add 50 ml of alcoholic KOH soln, 31.24, and 25 ml of alcoholic  $\text{AgNO}_3$  soln (0.2 g in 50 ml of alcohol), and connect with reflux condenser. Boil gently 1.5 hours, adding at intervals thru condenser 25 ml more of the alcoholic  $\text{AgNO}_3$  soln. Cool, and transfer contents of flask to large evaporating dish. Dilute to 200 ml and decant into a beaker, washing the sediment of  $\text{Ag}_2\text{O}$  with  $\text{H}_2\text{O}$  by decantation. Boil the soln 5 min. with 1 g of Zn dust to clarify; filter into another beaker, washing thoroly with  $\text{H}_2\text{O}$ , and add  $\text{HNO}_3$  to decided acidity and 0.1 *N* aqueous  $\text{AgNO}_3$  soln to complete precipitation. When the  $\text{AgBr}$  has agglutinated filter on weighed Gooch crucible, wash with  $\text{H}_2\text{O}$  and alcohol, dry at  $100^\circ$ , and weigh. Run a blank on the reagents used. Correct for the presence of halogens if necessary. Weight of  $\text{AgBr} \times 1.23 =$  weight of monobromated camphor.

**METHENAMINE (HEXAMETHYLENETETRAMINE) IN TABLETS (#7)—OFFICIAL,  
FIRST ACTION**

**39.56**

**REAGENT**

*Modified Nessler reagent.*—(1) Dissolve 10 g of  $\text{HgCl}_2$ , 30 g of KI, and 5 g of acacia in 200 ml of  $\text{H}_2\text{O}$ , and filter thru cotton; (2) dissolve 15 g of NaOH in 100 ml of  $\text{H}_2\text{O}$ ; (3) mix 20 ml of soln (1) with 10 ml of soln (2).

**39.57**

**PREPARATION OF SAMPLE.**—See 39.1

**39.58**

**DETERMINATION**

Weigh 0.5 g of powder into round-bottomed flask, and add 100 ml of  $\text{H}_2\text{O}$  and 25 ml of HCl (1+2.5). Connect with reflux condenser (preferably of worm type) and boil gently 15 min. Cool, wash condenser tube with a little  $\text{H}_2\text{O}$ , and transfer contents of flask to 250 ml volumetric flask, finally diluting to mark. Chill 30 ml of the Nessler reagent and add a 10 ml aliquot of the hydrolyzed soln of sample. Wash neck of container with jet of  $\text{H}_2\text{O}$  and allow to stand at least 1 min. Add 10 ml of acetic acid (1+1.5) in such manner that inside of neck is completely washed by the reagent, mix quickly and thoroly by rotating and tilting flask, and immediately add from a buret 20 ml of 0.1 *N* I soln. Titrate excess I with 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3$ , adding 5–10 drops of starch indicator, 6.3(e), toward end of operation, to disappearance of blue color. Final color of soln is a pale straw-green. If preferred, end point may be determined by reappearance of a faint blue color by addition of a drop of the I soln. 1 ml of 0.1 *N* I = 0.00117 g of methenamine.

**METHYLENE BLUE (METHYLTHIONINE CHLORIDE) (#8)—OFFICIAL**

**39.59**

**PREPARATION OF SAMPLE.**—See 39.1

**39.60**

**PREPARATION OF SOLUTION**

(a) *Foreign material absent.*—Weigh into 50 ml beaker 0.1–0.14 g of the powdered sample, 39.1, and transfer to 200 ml volumetric flask with 100–140 ml of  $\text{H}_2\text{O}$ . Dissolve completely by heating on steam bath, with frequent shaking, for 30 min.

(b) *Oils or water-insoluble material present.*—Transfer to 150 ml beaker weighed quantity of prepared sample, 39.1, corresponding to 0.1–0.14 g of methylene blue. Add 15 ml of  $\text{CCl}_4$ , warm on steam bath a few minutes, and stir with glass rod to dissolve oils. Transfer to 100 ml separator, using ca 50 ml of hot  $\text{H}_2\text{O}$  and a little  $\text{CCl}_4$  if necessary. Cool, shake, and allow to separate. Transfer the  $\text{CCl}_4$  with the undissolved material into second separator for further treatment. (A clear aqueous soln of the dye should now remain in the first separator. If not clear, extract with



another 15 ml portion of  $\text{CCl}_4$ , transferring in similar manner any remaining insoluble material to second separator.) Add ca 10 ml of  $\text{CCl}_4$  to second separator and remove the methylene blue by shaking vigorously with 20–40 ml portions of  $\text{H}_2\text{O}$  until practically no more dye is extracted. (A few drops of acetic acid hastens this extraction.) To the aqueous extracts in 400 ml beaker add main soln from first separator, cover with inverted watch-glass on glass rods, and evaporate to volume of ca 50 ml. Proceed as directed under (c). The  $\text{CCl}_4$  soln may be reserved for qualitative tests for oils.

(c) *Water-soluble material present.*—Use either the aqueous soln from (b), or a weighed portion of the sample corresponding to 0.1–0.14 g of methylene blue, dissolved by heating on steam bath in 150 ml beaker with ca 50 ml of  $\text{H}_2\text{O}$  for 30 min. with occasional shaking. Transfer to 100 ml separator, keeping volume as small as possible. Extract with  $\alpha$ -dichlorhydrin, using 10, 5, 3, and 2 ml portions. Combine the dichlorhydrin extracts in 200–300 ml separator, add 3 or 4 times their volume of  $\text{CCl}_4$ , and extract dye with  $\text{H}_2\text{O}$  by repeated vigorous shaking with 30–50 ml portions. (A few drops of acetic acid hastens the removal.) From the combined aqueous extracts remove any traces of dichlorhydrin by shaking once with ca 15 ml of  $\text{CCl}_4$ , which is drawn off after settling 5–10 min. Evaporate aqueous extracts to ca 50 ml over a flame, covering beaker as in (b) with inverted watch-glass. Transfer to 200 ml volumetric flask. Dissolve completely by heating on steam bath with frequent shaking for 30 min.

## 39.61

## DETERMINATION

Conduct a blank in same manner as the determination, including filtration. Cool the soln from 39.60(a) or (c), add 50 ml of acetic acid, shake thoroly, and allow to stand at least 25 min. Add from buret a total of 30 ml of 0.2 *N* I, adding first 10 ml by fast drops with constant rotating of the flask and the remaining 20 ml at full speed, and continue the shaking. Stopper flask and allow to stand 50 min., shaking thoroly 5 or 6 times during interval. Dilute to mark with  $\text{H}_2\text{O}$ , shake, and let stand 10 min. longer. Filter rapidly thru dry, folded, 12 cm filter paper. Titrate 100 ml aliquot with 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3$ , with or without starch indicator as desired. Correct for number of ml required to titrate blank run in same way. 1 ml of 0.2 *N* I = 0.01495 g of methylene blue ( $\text{C}_{16}\text{H}_{18}\text{N}_3\text{ClS} \cdot 3\text{H}_2\text{O}$ ) or 0.01324 g of anhydrous methylene blue ( $\text{C}_{16}\text{H}_{18}\text{N}_3\text{ClS}$ ).

## NITROGLYCERIN (29)—OFFICIAL, FIRST ACTION

## 39.62

## REAGENT

*Alcoholic potassium hydroxide soln.*—Dissolve 15 g of KOH in alcohol and dilute to 100 ml.

## 39.63

## APPARATUS

(a) *Connecting bulb.*—Hopkins style, ca 7.6 cm (3") in diam. This style has long inlet tube with opening on side of tube.

(b) *Condenser.*—Water-cooled, length 56 cm (22"), and preferably of Pyrex glass.

(c) *Adapter tube.*—Approximately 2.25 cm ( $\frac{1}{4}$ ") in diam. at top and with narrow outlet.

(d) *Scrubber-trap.*—Any efficient trap in which all vapor is washed thoroly with  $\text{H}_2\text{O}$  before it leaves distilling flask (see Fig. 61).

## 39.64

## DETERMINATION

*Method I*

(a) Place in 50 ml beaker sufficient quantity of weighed sample to yield ca 0.0324 g of nitroglycerin. If sample consists of tablets, count those taken; if of powdered material, mix thoroly before weighing. Add 10 ml of ether, and to facilitate extraction reduce tablets to fine powder by means of glass stirring rod having flattened end. Stir thoroly. Decant the ether thru dry 7 cm quantitative filter paper into 250 ml beaker containing 10 ml of alcohol. Hold filter paper in place in funnel with the stirring rod and pour ether down rod. Make four additional extractions in same way. Dissolve ether-insoluble residue in small quantity of  $H_2O$ , transfer soln to separator, and extract twice with 10 ml portions of ether. Filter these extracts, add them to first extracts, and evaporate combined solns to volume of ca 10 ml by means of fan.

(b) Transfer the alcoholic soln containing the nitroglycerin to 800 ml Kjeldahl flask, rinsing beaker first with 10 ml of alcohol and then with a little  $H_2O$ . Dilute to ca 300 ml with recently boiled and cooled  $NH_4$ -free  $H_2O$  and place flask on wire gauze with asbestos center. Add 2 g of Devarda alloy (by means of funnel), ca 4 cm of heavy (ca 16 gage) Al wire, and 10–15 ml of the alcoholic KOH soln. Immediately after adding the alkali, place a little  $H_2O$  in the scrubber trap (A and B), and insert into neck of flask the rubber stopper carrying scrubber trap and connecting bulb. Connect outlet tube of connecting bulb with the water-cooled condenser, which has been fixed in an upright position and fitted with an adapter tube dipping to bottom of 500 ml Erlenmeyer flask containing a measured volume (ca 25 ml) of 0.02 *N* acid ( $HCl$  or  $H_2SO_4$ ) and 10–15 ml of  $H_2O$ , and so inclined that tip of adapter is submerged as far as practicable under surface of liquid in flask. Heat distillation flask ca 1 hour, using small flame and regulating heat so that rapid evolution of  $H$ —but no appreciable distillation—takes place. Gradually increase heat until distillation begins; when active foaming ceases, continue distillation with large flame until all but ca 40 ml of liquid in distilling flask has distilled over. Lower flame toward end of distillation to avoid cracking flask. Remove receiver containing distillate, add sufficient methyl red indicator, 39.8(b), to make soln red, and titrate excess acid with 0.02 *N* NaOH soln. From difference between this excess and quantity added, after making such correction as may be shown to be necessary by blank test with same quantity of reagents distilled in same manner, calculate percentage of nitroglycerin in sample. 1 ml of 0.02 *N* acid = 0.001514 g of nitroglycerin.

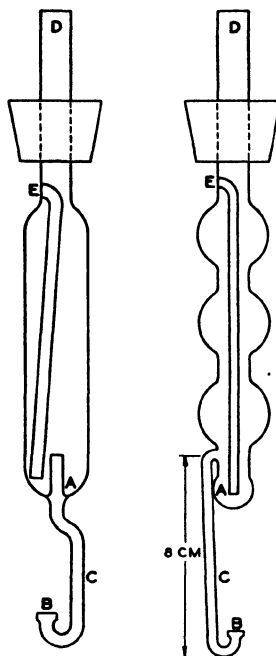


FIG. 61.—SCRUBBER TRAP FOR AMMONIA DISTILLATION

## 39.65

*Method II*

Place in glass-stoppered Erlenmeyer flask sufficient sample, accurately weighed, to yield ca 0.0648 g (1 grain) of nitroglycerin. If sample consists of tablets, count those taken; if of powdered material, mix thoroly before weighing portion taken

for analysis. Add 50 ml of alcohol by means of pipet. To facilitate extraction reduce tablets to fine powder with glass stirring rod flattened at one end. Stopper flask and shake. Allow mixture to settle, transfer 25 ml aliquot of clear soln to 800 ml Kjeldahl distilling flask, dilute to ca 300 ml with  $\text{NH}_3$ -free  $\text{H}_2\text{O}$ , and proceed as directed in 39.64(b).

#### ELIXIR OF THREE BROMIDES (30)—TENTATIVE

##### 39.66

##### PREPARATION OF DILUTION

Dilute 25 ml of the elixir to 250 ml. Measure aliquot of this dilution at the original temp.

##### 39.67

##### DETERMINATION

(a) *Ammonium bromide*.—Transfer 50 ml aliquot to Kjeldahl flask provided with trap and condenser, add 150 ml of  $\text{H}_2\text{O}$  and an excess of 10%  $\text{NaOH}$  soln (ca 5 ml). Distil the  $\text{NH}_3$  into excess of 0.1  $N$   $\text{H}_2\text{SO}_4$  (ca 50 ml), and titrate excess acid with 0.1  $N$   $\text{NaOH}$ , using methyl red indicator, 39.8(b).

$$1 \text{ ml of } 0.1 \text{ } N \text{ } \text{H}_2\text{SO}_4 = 0.00980 \text{ g of } \text{NH}_4\text{Br.}$$

(b) *Potassium bromide*.—Evaporate 10 ml aliquot and ignite at dull red temp. (ca  $525^\circ$ ). Treat residue with hot  $\text{H}_2\text{O}$ , filter, and wash into porcelain evaporating dish. Convert the bromides to chlorides by treating residue with two portions of  $\text{Cl-H}_2\text{O}$ , evaporating between additions, and proceed as directed under 12.17, beginning "acidify with a few drops of  $\text{HCl}$ ."

$$\text{K}_2\text{PtCl}_6 \times 0.4896 = \text{KBr.}$$

(c) *Sodium bromide*.—Transfer 5 ml aliquot to suitable beaker and proceed as directed under 12.21, beginning "Add 100 ml of the  $\text{Mg}$  uranyl acetate soln."

$$\text{Weight of Na-Mg-uranyl acetate} \times 0.0685 = \text{NaBr.}$$

(d) *Total bromine*.—Transfer 10 ml aliquot to flask and add slowly and with agitation 30 ml of 0.1  $N$   $\text{AgNO}_3$ , 2 ml of  $\text{HNO}_3$ , and 2 ml of  $\text{FeNH}_4(\text{SO}_4)_2$  soln. Titrate excess  $\text{AgNO}_3$  with 0.1  $N$   $\text{NH}_4\text{SCN}$ .

$$1 \text{ ml of } 0.1 \text{ } N \text{ } \text{AgNO}_3 = 0.00799 \text{ g of Br.}$$

#### ELIXIR OF FIVE BROMIDES (30)—TENTATIVE

##### 39.68

##### PREPARATION OF DILUTION

Transfer 50 ml of the elixir to 1 liter volumetric flask, dilute to mark, and mix. Measure aliquots of this dilution at original temp.

##### 39.69

##### DETERMINATION

(a) *Ammonium bromide*.—Introduce a 200 ml aliquot of the dilution into a Kjeldahl flask, and add small piece of paraffin and an excess of 10%  $\text{NaOH}$  soln (ca 5 ml). Distil the  $\text{NH}_3$  into excess of standard acid, 40 ml of 0.1  $N$  usually being sufficient. Titrate excess acid with 0.1  $N$   $\text{NaOH}$ , using methyl red indicator, 39.8(b).

$$1 \text{ ml of } 0.1 \text{ } N \text{ acid} = 0.00980 \text{ g of } \text{NH}_4\text{Br.}$$

(b) *Calcium bromide*.—Pipet a 100 ml aliquot of the dilution into casserole or Pt dish and evaporate to dryness. Ignite at dull red (ca  $525^\circ$ ) until organic matter is thoroly charred. Add 5 ml of 10%  $\text{HCl}$  to dissolve  $\text{Ca}$  salts, filter, and wash well with hot  $\text{H}_2\text{O}$ . Return filter and unoxidized  $\text{C}$  to casserole or dish and ignite at moderate temp. until residue is white. Treat residue with 5 ml of 10%  $\text{HCl}$ , filter, and wash with hot  $\text{H}_2\text{O}$ , combining filtrates.

Determine Ca as directed in 12.12, and reserve filtrate for determination of Na, K, and Li. If 0.1 *N* KMnO<sub>4</sub> is used, 1 ml = 0.0100 g of CaBr<sub>2</sub>.

(c) *Lithium bromide*.—Dilute filtrate and washings from Ca determination to 200 ml and mix. Evaporate a 100 ml aliquot to dryness and drive off all NH<sub>4</sub> salts by heating to faint redness (ca 525°) in Pt dish. Treat residue with a little H<sub>2</sub>O, filter into Pt dish, add a few ml of HCl, and evaporate to dryness.

Complete conversion of alkali bromides to chlorides by treating residue with Cl-H<sub>2</sub>O and evaporating to dryness. Repeat addition and evaporation of Cl-H<sub>2</sub>O twice more, or until there is no apparent darkening of soln due to liberation of Br. Proceed as directed in 37.66, and 37.67, beginning "Dissolve mixed chlorides in hot H<sub>2</sub>O, filter, and wash." (Since Na and K are to be determined directly, it is not necessary to weigh the mixed chlorides.)

$$\text{Li}_2\text{SO}_4 \times 1.5801 = \text{LiBr.}$$

(d) *Sodium bromide*.—Remove the combined KCl and NaCl from the Gooch crucible by washing with hot H<sub>2</sub>O, make to 50 ml, and use a 5 ml aliquot for the determination of Na. Proceed as directed in 12.21, beginning "Add 100 ml of the Mg uranyl acetate soln." Calculate to NaBr, using factor 0.0685.

(e) *Potassium bromide*.—Use a 25 ml aliquot of the soln of KCl and NaCl and proceed as directed in 37.67, beginning "Add sufficient Pt soln, 2.40(b), to convert the KCl and NaCl to K<sub>2</sub>PtCl<sub>6</sub> and Na<sub>2</sub>PtCl<sub>6</sub> and evaporate to dryness." Calculate to KBr, using factor 0.4896.

(f) *Total bromine*.—Transfer 20 ml of the dilution to 500 ml flask. Add 100 ml of H<sub>2</sub>O, 2 ml of HNO<sub>3</sub>, and an excess of 0.1 *N* AgNO<sub>3</sub> (usually 30 ml). Titrate the excess AgNO<sub>3</sub> with 0.1 *N* NH<sub>4</sub>SCN, using Fe alum indicator.

$$1 \text{ ml of } 0.1 \text{ } N \text{ AgNO}_3 = 0.00799 \text{ g of Br.}$$

### 39.70

#### TERPIN HYDRATE AND CODEINE IN ELIXIRS (51)—TENTATIVE

(a) *Terpin hydrate*.—Measure 10 ml sample from small buret (allow buret to drain 5 min.) into separator. Add 10 ml of H<sub>2</sub>O and 5 ml of 10% H<sub>2</sub>SO<sub>4</sub>, and immediately extract with 25 ml of petroleum benzene. Drain aqueous layer into second separator. Wash the petroleum benzene twice with 2 ml portions of H<sub>2</sub>O and add washings to aqueous layer. Discard the petroleum benzene. Completely extract aqueous soln with CHCl<sub>3</sub>-alcohol solvent (95+5), (7 extractions of 20, 10, 10, 10, 10, 10, 10 ml should be sufficient). Make an additional extraction and evaporate to dryness to test for complete extraction. Combine CHCl<sub>3</sub>-alcohol extracts and wash with 5 ml of H<sub>2</sub>O. Filter extract thru pledget of cotton, previously wet with the solvent, into tared dish. Place dish in desiccator (or similar apparatus) prepared according to Fig. 62. Adjust vacuum so incoming air just ripples surface of liquid, and evaporate to apparent dryness. Weigh residue and report as g/100 ml of terpin hydrate.

(b) *Codeine*.—Measure 25 ml sample into a separator, add 25 ml of H<sub>2</sub>O and 1 ml of NH<sub>4</sub>OH soln (10% NH<sub>3</sub>) and completely extract the alkaloid with CHCl<sub>3</sub> (6 extractions of 25, 20, 15, 10, 10, and 10 ml should be sufficient). Combine extracts in second separator and completely extract the codeine with 10% H<sub>2</sub>SO<sub>4</sub> (4 extractions of 15, 10, 10, and 10 ml should be sufficient). Wash combined acid extracts with 10 ml of CHCl<sub>3</sub> and discard solvent. Make acid soln ammoniacal and extract 5 times with CHCl<sub>3</sub>, using 30, 20, 20, 10, and 5 ml. Test for complete extraction of the alkaloid. (Make additional extraction with 10 ml of CHCl<sub>3</sub>; evaporate solvent in separate beaker and dissolve residue in a few drops of methyl alcohol; add a drop of methyl red indicator, 39.8(b), and dilute with 20 ml of H<sub>2</sub>O, carbonate free. Yellow color indicates incomplete extraction. Titrate, and add quantity thus ob-

tained to total.) Combine the  $\text{CHCl}_3$  extractions in second separator, into stem of which is inserted pledget of cotton wet with  $\text{CHCl}_3$ . Wash combined extractions with 1 ml of  $\text{H}_2\text{O}$  containing 1 drop of  $\text{NH}_4\text{OH}$  and evaporate on water bath, using electric fan to prevent decrepitation of residue. When dry, remove immediately and complete determination by one of following procedures:

(1) To alkaloidal residue add 2–3 ml of methyl alcohol, cover beaker with watch-glass, and heat on steam bath until residue, including any portions thereof that

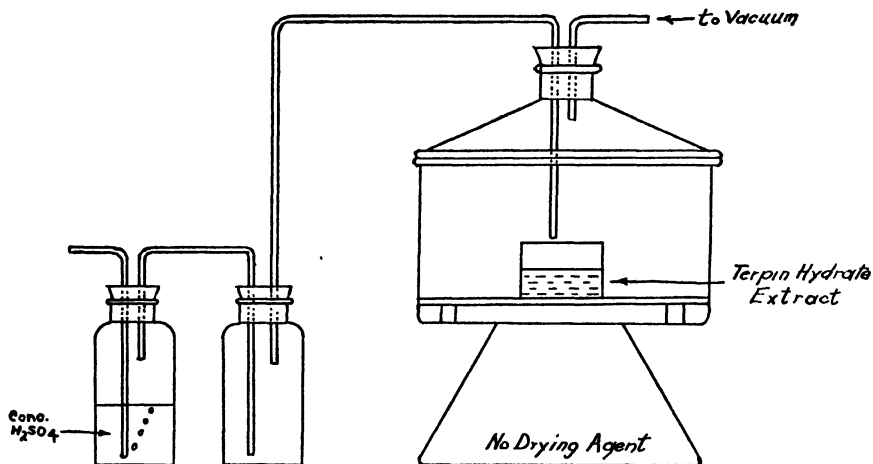


FIG. 62.—APPARATUS USED IN DETERMINATION OF TERPIN HYDRATE AND CODEINE IN ELIXIR

may adhere to upper part of beaker, is completely dissolved. Add 2 drops of methyl red indicator 39.8(b), and, without dilution with  $\text{H}_2\text{O}$ , titrate carefully with 0.02  $N$   $\text{H}_2\text{SO}_4$  to a faint pink, avoiding an excess. Cover beaker and digest on steam bath until all particles are completely dissolved. If more than 2 ml of alcohol is added, evaporate excess. Cool, and dilute with 50 ml of boiled  $\text{H}_2\text{O}$  (soln should now be yellow). Finish titration with the standard acid to faint red.

(2) Dissolve residue in 2–3 ml of methyl alcohol on steam bath. Add 2 drops of the methyl red indicator and then add from buret 5–10 ml excess of 0.02  $N$   $\text{H}_2\text{SO}_4$ , noting total quantity used. Cover beaker with watch-glass and heat on steam bath until residue, including any portions thereof that may adhere to upper part of beaker, is completely dissolved. Dilute with 50 ml of cold, previously boiled  $\text{H}_2\text{O}$ . Titrate back with the 0.02  $N$   $\text{NaOH}$ . The  $\text{H}_2\text{O}$  and alkali should be sufficiently free from carbonates to insure a sharp end point with methyl red. Report as g/100 ml of codeine hydrate. 1 ml of 0.02  $N$   $\text{H}_2\text{SO}_4 = 0.00635$  g of  $\text{C}_{18}\text{H}_{21}\text{O}_3\text{N} \cdot \text{H}_2\text{O}$ .

### 39.71

#### SULFONAL AND TRIONAL (32)—OFFICIAL, FIRST ACTION

Mix ca 0.5 g of sample with pure, clean sea sand and place mixture in Knorr tube containing half-inch layer of asbestos. Using bell jar and vacuum, extract mixture with 10 portions of 10 ml each of ether, mixing sample with sand by means of glass rod before each addition of ether. Collect ether extractions in tared flask, distil off bulk of ether, and allow remaining solvent to evaporate spontaneously,

rotating flask to aid evaporation. Dry residue in desiccator over  $\text{H}_2\text{SO}_4$  18 hours and weigh. Identify residue by means of mixed melting point.

If desired, extraction may be made in suitable automatic apparatus (Fig. 63).

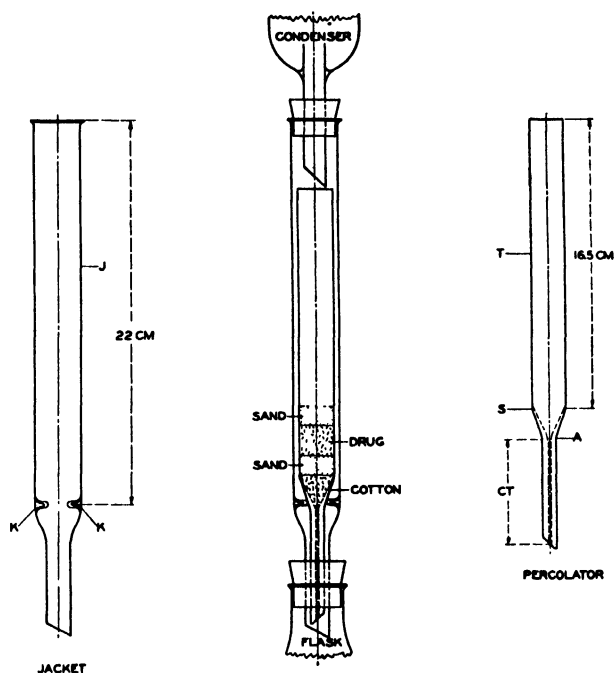


FIG. 63.—AUTOMATIC PERCOLATOR

### 39.72 ACONITINE IN ACONITE ROOT—QUALITATIVE TEST (35)—TENTATIVE

Crush and macerate the aconite root in a mortar with 15 ml of  $\text{H}_2\text{O}$  and transfer to extraction tube (Mojonnier type, Fig. 64). Add 5 ml of  $\text{NH}_4\text{OH}$  (10%  $\text{NH}_3$ ) and extract 2 or 3 times with 15 ml portions of ether. Transfer ethereal extract to separator and wash with  $\text{H}_2\text{O}$ . Extract washed, ethereal extract with 2 or 3 ml of 0.02  $N$   $\text{H}_2\text{SO}_4$ . Test aqueous layer with methyl red indicator; if alkaline, discard aqueous layer. Continue to extract with 2 or 3 ml of 0.02  $N$   $\text{H}_2\text{SO}_4$  until aqueous layer remains acid to methyl red indicator. Test the slightly acid aqueous layer for aconitine by following method:

In small test tube add 1 or 2 drops of 5%  $\text{Na}_2\text{CO}_3$  soln to 1 or 2 ml of the slightly acid, aqueous soln. Heat to  $60^\circ$ , stirring with thermometer. Cool, and transfer a few drops of the liquid to microscope slide and examine crystals. Irregular hexagonal plates are formed by aconitine. Most characteristic crystals of aconitine are formed in a concentration of 1/1000 or less.

### 39.73 ARECOLINE HYDROBROMIDE (34)—TENTATIVE

Accurately weigh sufficient sample to insure 0.1–0.15 g of arecoline hydrobromide. Rinse sample into 125 ml separator, stem of which holds a fairly tight-fitting cotton pledget moistened with  $\text{CHCl}_3$ . Add enough  $\text{H}_2\text{O}$  to contents of separator to make total volume of 20 ml (sufficient to dissolve the arecoline hydrobromide); to the

soln add solid  $\text{NaHCO}_3$  until small quantity remains undissolved. Extract with  $\text{CHCl}_3$ , using 30, 25, 20, 15, 10 ml portions, and test for complete extraction. Draw off extract into 250 ml tall-form beaker or (preferably) 500 ml Erlenmeyer flask. To beaker or flask add 35 ml of 0.02  $N$   $\text{H}_2\text{SO}_4$ , and evaporate the  $\text{CHCl}_3$  on steam bath, using glass beads or a stirring rod to prevent superheating. When  $\text{CHCl}_3$  has evaporated, cool acid soln and titrate with 0.02  $N$   $\text{NaOH}$ , using 2 drops of methyl red indicator, 39.8(b). 1 ml of 0.02  $N$   $\text{H}_2\text{SO}_4 = 0.00472$  g of  $\text{C}_8\text{H}_{13}\text{O}_2\text{N} \cdot \text{HBr}$ .

## 39.74

## ATROPINE IN TABLETS (55)—OFFICIAL

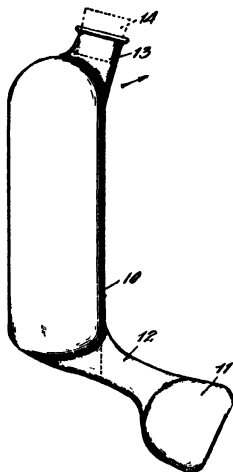


FIG. 64.—MOJONNIER-TYPE EXTRACTION TUBE

Count and weigh sufficient tablets to yield ca 0.065 g of alkaloid and introduce directly into small separator. Dissolve in 5–20 ml of  $\text{H}_2\text{O}$  and add 1 ml of  $\text{NH}_4\text{OH}$ . Add an equal volume of  $\text{CHCl}_3$ , agitate, and allow to stand until separation is complete. Draw off  $\text{CHCl}_3$  layer into second separator and repeat extraction with fresh portions of solvent until alkaloid is completely removed. After combining all the fractions, wash combined  $\text{CHCl}_3$  solns by agitation with 5 ml of  $\text{H}_2\text{O}$  and allow to clear. Introduce pledget of absorbent cotton into stem of separator and draw off  $\text{CHCl}_3$  soln into small beaker. Add 10 ml of  $\text{CHCl}_3$ , agitate, and when the  $\text{H}_2\text{O}$  has entirely risen to surface draw off  $\text{CHCl}_3$  into beaker. Wash outer surface of stem of separator with a little  $\text{CHCl}_3$ , adding washing to beaker. Evaporate soln on steam bath to ca 5 ml. Add a measured excess volume of 0.02  $N$   $\text{H}_2\text{SO}_4$ , and continue evaporation until odor of  $\text{CHCl}_3$  has disappeared. Cool soln and titrate back with 0.02  $N$   $\text{NaOH}$ , using 1 drop of methyl red indicator. 1 ml of 0.02  $N$   $\text{H}_2\text{SO}_4 = 0.00578$  g of atropine or 0.00695 g of atropine sulfate.

## 39.75

## BENZEDRINE (56)—TENTATIVE

Place 30 ml of 10%  $\text{NaOH}$  soln in 125 ml separator and add an aliquot of the benzedrine soln containing 50–100 mg of benzedrine. Add 5 drops of benzoyl chloride and shake vigorously. Allow to stand with occasional shaking for 10 min. Again add 5 drops of benzoyl chloride and again shake the separator occasionally for 10 min. Add a third 5-drop portion of benzoyl chloride, shake, and test reaction of soln with litmus paper. If necessary, add more of the  $\text{NaOH}$  soln to maintain alkalinity thruout the determination. Allow to stand 2 hours with occasional shaking. (At room temp. complete hydrolysis of the benzoyl chloride requires considerable time.) Extract soln with three 20 ml portions of washed  $\text{CHCl}_3$ . Test for complete extraction, using a 10 ml portion of washed  $\text{CHCl}_3$ . Combine  $\text{CHCl}_3$  extracts in second separator and wash with 5 ml of  $\text{H}_2\text{O}$ . Drain the  $\text{CHCl}_3$  thru a pledget of cotton into a tared beaker. Carefully evaporate  $\text{CHCl}_3$  almost to dryness and add 2–3 ml of anhydrous ether. Carefully evaporate ether, finally reducing heat to avoid decrepitation. Allow beaker to attain constant weight in open air without further heating.  $\text{C}_{16}\text{H}_{17}\text{ON} \times 0.565 = \text{C}_9\text{H}_{13}\text{N}$ .

## COCAINE (57)

## 39.76

## REAGENT

*Mayer reagent (mercuric-potassium iodide soln).*—Dissolve 1.36 g of  $\text{HgCl}_2$  in 60 ml of  $\text{H}_2\text{O}$  and 5 g of  $\text{KI}$  in 10 ml of  $\text{H}_2\text{O}$ ; mix these two solns and dilute to 100 ml.

## 39.77

*Method I—Official*

Weigh accurately sufficient uniformly mixed sample to represent 0.1–0.2 g of the alkaloid. Transfer to small separator and dissolve in minimum quantity of  $\text{H}_2\text{O}$ . Make soln slightly alkaline with  $\text{NH}_4\text{OH}$  and extract with successive small portions of peroxide-free ether until alkaloid is completely removed from aqueous soln, using the Mayer reagent for test. Combine ether extracts, remove greater part of ether by evaporation on steam bath, and allow remainder to evaporate spontaneously at room temp. Dissolve residue in a few ml of neutral alcohol, add 20 ml of 0.05  $N$   $\text{H}_2\text{SO}_4$ , and titrate excess of acid with 0.02  $N$   $\text{NaOH}$ , using methyl red indicator, 39.8(b). 1 ml of 0.05  $N$   $\text{H}_2\text{SO}_4 = 0.01699$  g of cocaine hydrochloride,  $\text{C}_{17}\text{H}_{21}\text{O}_4\text{N} \cdot \text{HCl}$ .

## 39.78

*Method II—Tentative*

Weigh accurately sufficient uniformly mixed sample to represent ca 0.2 g of the alkaloid. Dissolve in 20 ml of cold  $\text{H}_2\text{O}$ , add 2 drops of 10%  $\text{HCl}$ , and transfer to separator. Make alkaline to litmus with freshly prepared saturated  $\text{NaHCO}_3$  soln and shake out to exhaustion with petroleum benzine (four 20 ml portions are usually sufficient). Run combined extracts thru plug of absorbent cotton into separator and wash cotton with petroleum benzine. Add decided excess of 0.02  $N$   $\text{H}_2\text{SO}_4$ , accurately measured, and shake vigorously several minutes. Separate the 2 layers and wash the petroleum benzine with two 10 ml portions of  $\text{H}_2\text{O}$ , adding washings to acid soln. Titrate excess acid with 0.02  $N$  alkali, using methyl red indicator, and reserve the titrated soln for the check determination described below. 1 ml of 0.02  $N$   $\text{H}_2\text{SO}_4 = 0.006793$  g of cocaine hydrochloride,  $\text{C}_{17}\text{H}_{21}\text{O}_4\text{N} \cdot \text{HCl}$ .

As a check, add 10 ml of 2.5  $N$   $\text{NaOH}$  soln to the titrated alkaloidal soln and evaporate on steam bath to ca 10 ml. Cool, transfer soln to separator, and acidify with 10%  $\text{HCl}$ . Extract acid soln completely with successive portions of  $\text{CHCl}_3$ . Run combined extracts thru plug of absorbent cotton and wash cotton well with  $\text{CHCl}_3$ . Allow the  $\text{CHCl}_3$  soln to evaporate spontaneously in weighed beaker, dry residue in vacuum desiccator 2 hours, and weigh. From weight of benzoic acid found calculate its equivalent of cocaine hydrochloride.  $\text{C}_6\text{H}_5\text{COOH} \times 2.782 = \text{C}_{17}\text{H}_{21}\text{O}_4\text{N} \cdot \text{HCl}$ . (If desired, the benzoic acid may be determined by titration.)

**EMETINE HYDROCHLORIDE IN TABLETS (38)—OFFICIAL**

## 39.79

**PREPARATION OF SAMPLE—See 39.1**

## 39.80

**DETERMINATION**

Transfer to small separator sufficient powdered material, accurately weighed, to represent ca 0.1 g of the alkaloidal salt. Dissolve in minimum quantity of  $\text{H}_2\text{O}$  and add 5 ml of 4%  $\text{NaOH}$  soln. Extract with 30 ml of washed ether, draw off aqueous soln, and swirl separator to remove the  $\text{H}_2\text{O}$  from the sides. Wash the ether with 1 ml of  $\text{H}_2\text{O}$ , adding wash  $\text{H}_2\text{O}$  to aqueous soln. Decant the ether into third separator, washing mouth of separator with ether. Repeat extractions with 25, 20, 15, and 10 ml portions of ether or until extraction is complete, washing with 1 ml of  $\text{H}_2\text{O}$  each time, and combine ether extracts in third separator. Filter into beaker thru cotton previously wet with ether, finally wash separator with ether, evaporate bulk of ether on steam bath and complete evaporation at low temp.

To residue add 2 ml of neutral alcohol, cover beaker with watch-glass, and allow to reflux on steam bath a few minutes. Add a few drops of methyl red indicator, 39.8(b), and without dilution titrate with 0.02  $N$  acid to faint pink. Cover beaker and digest on steam bath until all particles are completely dissolved. Cool, and add



ca 30 ml of recently boiled  $\text{H}_2\text{O}$ . Finish titration with standard acid to faint red. 1 ml of 0.02  $N$   $\text{H}_2\text{SO}_4 = 0.00553$  g of emetine hydrochloride ( $\text{C}_{29}\text{H}_{46}\text{O}_4\text{N}_2 \cdot 2\text{HCl}$ ).

#### EPHEDRINE IN INHALANTS (39)—OFFICIAL, FIRST ACTION

##### 39.81

##### DETERMINATION

Weigh accurately into small tared beaker, 5–10 g of sample. Add 10 ml of 2%  $\text{H}_2\text{SO}_4$ , stir, and allow mixture to stand ca 15 min. Transfer to small separator, rinsing beaker with small portions of ether. Shake gently, and transfer acid layer to second separator. Shake with 3 successive 10 ml portions of 2%  $\text{H}_2\text{SO}_4$ , rinsing beaker with ether each time. Test for complete removal of alkaloid.

Neutralize the combined acid solns in separator with  $\text{NH}_4\text{OH}$ , and add 5 ml in excess. Extract soln with 30 ml of washed ether (automatic extractor optional), transfer aqueous layer to second separator, and wash ethereal extract with 1 ml of  $\text{H}_2\text{O}$ , adding washings to main aqueous soln. Swirl the ether in order to remove  $\text{H}_2\text{O}$  adhering to side of separator. After all  $\text{H}_2\text{O}$  has been removed, filter mixture into an Erlenmeyer flask thru pledget of cotton, wet with ether, inserted in a small funnel. Repeat extraction with liberal portions of washed ether at least 4 times, or until the alkaloid is removed completely, washing each portion with the same 1 ml of  $\text{H}_2\text{O}$ . Evaporate the ether to a volume of 10 ml by aid of current of air. Add bromothymol blue indicator, 6.117(e), a measured excess of 0.02  $N$   $\text{H}_2\text{SO}_4$ , and ca 40 ml of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ ; cover with watch-glass, heat on steam bath in order to dissolve alkaloid adhering to sides of flask, and evaporate all the ether. Cool, and titrate excess acid with 0.02  $N$   $\text{NaOH}$ , using indicator standard, pH 6.0, for comparison. 1 ml of 0.02  $N$   $\text{H}_2\text{SO}_4 = 0.00330$  g of ephedrine.

#### EPHEDRINE IN TABLETS (40)—OFFICIAL, FIRST ACTION

##### 39.82

##### PREPARATION OF SAMPLE—See 39.1

##### 39.83

##### DETERMINATION

Weigh accurately quantity of the powdered material equal to ca 0.12 g of the alkaloidal salt, and transfer to separator. Dissolve in minimum quantity of  $\text{H}_2\text{O}$ , then add 5 ml of  $\text{NH}_4\text{OH}$ . Extract soln with 30 ml of washed ether. Transfer aqueous layer to second separator. Wash ether extract with 1 ml of  $\text{H}_2\text{O}$ , adding washings to main aqueous soln. Swirl the ether in order to remove  $\text{H}_2\text{O}$  adhering to side of separator. After all  $\text{H}_2\text{O}$  has been removed, filter into beaker thru pledget of cotton, wet with ether, inserted in small funnel. Repeat extraction with liberal portions of ether at least 4 times, or until alkaloid is completely removed, washing each portion with 1 ml of  $\text{H}_2\text{O}$ . Evaporate ether to volume of ca 10 ml by aid of current of air and proceed as directed in 39.81, beginning "Add bromothymol blue." 1 ml of 0.02  $N$  acid = 0.00403 g of ephedrine hydrochloride, 0.00428 g of ephedrine sulfate, and 0.00330 g of ephedrine.

#### HOMATROPINE IN TABLETS (41)—OFFICIAL

##### 39.84

##### REAGENTS

(a) *Iodine soln.*—See 39.5(b).

(b) *Methyl red indicator.*—See 39.8(b).

39.85

## PREPARATION OF SAMPLE—See 39.1

39.86

## DETERMINATION

Weigh accurately a quantity of sample equal to ca 0.130 g of the alkaloidal salt, and transfer to separator. Dissolve in 10–20 ml of  $H_2O$  and add 2 ml of  $NH_4OH$ . Add ca 20 ml of  $CHCl_3$ , agitate, and allow to stand until separation is complete. Draw off  $CHCl_3$  layer into second separator and repeat extraction with fresh portions of solvent until alkaloid is completely removed (5 extractions usually suffice). Test for complete removal with the I reagent. After combining all fractions, wash  $CHCl_3$  solns by agitation with 5 ml of  $H_2O$  and allow to settle. Filter  $CHCl_3$  soln thru cotton into small beaker. Wash aqueous soln with 10 ml of  $CHCl_3$ ; draw off the  $CHCl_3$  and filter into the beaker. Wash outer surface of stem of separator and funnel and its stem with a little  $CHCl_3$ , adding washings to beaker. Evaporate soln on steam bath to ca 5 ml. Add measured excess of 0.02 N  $H_2SO_4$ . Place beaker in warm place and evaporate with aid of fan until odor of  $CHCl_3$  has disappeared. Cool the soln and titrate back with 0.02 N NaOH, using 1 drop of methyl red indicator, 39.8(b). 1 ml of 0.02 N  $H_2SO_4$  = 0.00713 g of homatropine hydrobromide or 0.00626 g of homatropine hydrochloride.

## PROSTIGMINE (48)—TENTATIVE

39.87

Weigh a portion of powdered material equivalent to ca 0.06 g of prostigmine bromide into 500 ml Kjeldahl flask. Add 200 ml of  $H_2O$ , 25 ml of NaOH (1+1), a few crystals of  $Ba(OH)_2$  to prevent foaming, and several glass beads. Distil the dimethylamine formed by alkaline hydrolysis into 25 ml of 0.02 N  $H_2SO_4$ , collecting at least 150 ml of distillate. Titrate the excess  $H_2SO_4$  with 0.02 N NaOH, using methyl red indicator, 39.8(b). Each ml of 0.02 N  $H_2SO_4$  = 0.00606 g of prostigmine bromide.

## OPIUM ALKALOIDS AND THEIR DERIVATIVES

39.88

## APOMORPHINE IN TABLETS (49)—OFFICIAL

Weigh a number of tablets equivalent to ca 0.065 g (1 grain) of the alkaloid or of its salt and dissolve in 10 ml of  $H_2O$  in separator. Add 1 ml of freshly prepared saturated  $NaHCO_3$  soln and 25 ml of peroxide-free ether, and shake mixture. After separation, draw off lower layer into second separator and transfer ethereal layer to third separator. Extract mixture in second separator repeatedly with 15 ml portions of ether until alkaloid has been completely removed, using second and first separators alternately for the shaking, and collecting all ethereal soln in the third. Discard aqueous soln. Wash ethereal soln of alkaloid 3 times with 5 ml portions of  $H_2O$ , uniting aqueous washings in a clean separator. Extract these washings with a little fresh peroxide-free ether. Discard aqueous portion, wash ether with  $H_2O$ , discard washings, and add washed ether to main portion of ethereal soln. Add 20 ml of 0.02 N  $H_2SO_4$  to ethereal soln of alkaloid in separator and shake mixture thoroly. Transfer mixture to beaker, wash separator twice with 5 ml portions of  $H_2O$ , adding washings to acid liquid in beaker, and without delay evaporate ether at low temp., preferably on water bath with aid of blast of air. Titrate excess acid with 0.02 N NaOH, using one drop of methyl red indicator, 39.8(b). 1 ml of 0.02 N  $H_2SO_4$  = 0.00625 g of apomorphine hydrochloride,  $C_{17}H_{17}O_2N \cdot HCl \cdot \frac{1}{2}H_2O$ .

## CODEINE IN TABLETS (44)

39.89

*Qualitative Tests—Official*

(a) To the residue or tablet add  $\text{HNO}_3$ . A yellow color is produced.

(b) To 3 ml of an aqueous soln (1+200), add a few drops of 10%  $\text{K}_3\text{Fe}(\text{CN})_6$  soln and 1 or 2 drops of 10%  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  soln. A green color is produced.

39.90

*Quantitative Method—Official*

Transfer to small separator sufficient tablets, or powdered material equal to a multiple of average weight per tablet, to represent ca 0.15 g of the alkaloid. Dissolve in minimum quantity of  $\text{H}_2\text{O}$  (not over 5 ml) acidified with 2 drops of  $\text{HCl}$ . Add solid  $\text{NaHCO}_3$  until neutralized, then a slight excess, and extract 5 times with  $\text{CHCl}_3$ , using 30, 20, 20, 10, and 5 ml. Test for complete extraction of the alkaloid. (Make an additional extraction with 10 ml of  $\text{CHCl}_3$ , evaporate solvent in separate beaker, and dissolve residue in a few drops of methyl alcohol. Add a drop of methyl red indicator, 39.8(b), and dilute with 20 ml of  $\text{H}_2\text{O}$ , carbonate-free. Yellow color indicates incomplete extraction. Titrate, and add quantity thus obtained to total.) Combine  $\text{CHCl}_3$  extracts in second separator, into stem of which is inserted pledget of cotton wet with  $\text{CHCl}_3$ . Wash combined extracts with 1 ml of  $\text{H}_2\text{O}$  containing 1 drop of  $\text{NH}_4\text{OH}$  and proceed as directed under 39.96, beginning "Evaporate on water bath." 1 ml of 0.02  $N$   $\text{H}_2\text{SO}_4 = 0.00787$  g of codeine sulfate,  $(\text{C}_{18}\text{H}_{21}\text{O}_2\text{N})_2 \cdot \text{H}_2\text{SO}_4 \cdot 5\text{H}_2\text{O}$ , or 0.00849 g of codeine phosphate,  $\text{C}_{18}\text{H}_{21}\text{O}_3\text{N} \cdot \text{H}_3\text{PO}_4 \cdot 1\frac{1}{2}\text{H}_2\text{O}$ .

## DIACETYLMORPHINE (HEROINE) IN TABLETS (45)

39.91

*Qualitative Test—Official*

Heat ca 0.1 g with 1 ml of  $\text{H}_2\text{SO}_4$  and 1 ml of alcohol. Ethyl acetate, readily recognized by its odor, is formed.

39.92

*Quantitative Method—Official*

Weigh, and transfer directly to small separator a number of tablets representing ca 0.15 g of diacetylmorphine. Dissolve in 5 ml of  $\text{H}_2\text{O}$  containing 1 drop of acetic acid. Add 1 ml of  $\text{NH}_4\text{OH}$  and extract 5 times with  $\text{CHCl}_3$ , using 30, 20, 10, 10, and 5 ml, respectively. Combine  $\text{CHCl}_3$  extracts in second separator, into stem of which is inserted pledget of cotton wet with  $\text{CHCl}_3$ . Wash combined extracts with 1 ml of  $\text{H}_2\text{O}$  and proceed as directed under 39.70(b), beginning "evaporate on water bath." 1 ml of 0.02  $N$   $\text{H}_2\text{SO}_4 = 0.00847$  g of diacetylmorphine hydrochloride,  $\text{C}_{21}\text{H}_{23}\text{O}_5\text{N} \cdot \text{HCl} \cdot \text{H}_2\text{O}$ .

## MORPHINE IN TABLETS (46)

39.93

*Qualitative Tests—Official*

(a) To the residue or tablet add  $\text{HNO}_3$ . An orange-red color fading to yellow is produced.

(b) To an aqueous soln add a few drops of 10%  $\text{K}_3\text{Fe}(\text{CN})_6$  soln and then one drop of 10%  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  soln. Deep blue soln results; blue precipitate separates on standing.

(c) See Microchemical Tests, 39.244.

*Quantitative Method—Official*

39.94

## REAGENT

*Alkaline salt soln.*—Dissolve 30 g of  $\text{NaOH}$  in  $\text{H}_2\text{O}$ , dilute to 1 liter, add  $\text{NaCl}$  to saturation, and filter.

## 39.95

## PREPARATION OF SAMPLE

To ascertain variation in weight, weigh separately at least 20 tablets. Also weigh collectively a representative number of unbroken tablets and calculate average weight per tablet. To insure representative sampling in tablets containing more than  $\frac{1}{2}$  grain of alkaloid, pulverize ca 20 tablets, mix powder thoroly, and protect from moisture in well-stoppered bottle.

## 39.96

## DETERMINATION

Transfer to small separator sufficient tablets, or powdered material equal to a multiple of average weight per tablet, to represent ca 0.15 g of the alkaloid. Moisten with 5 ml of  $H_2O$ , shake gently, and then dissolve completely by adding 10 ml of the alkaline NaCl soln. (Excipients may not be completely soluble.) To the alkaline NaCl soln, add small piece of litmus paper and then HCl, dropwise, until neutral. Add 10 drops in excess. Add 5 ml of alcohol, carefully neutralize with  $NH_4OH$  dropwise, and then add 5 drops in excess. Invert separator and open stopcock to insure neutralization of residual acid. Immediately extract, at least 6 times, with  $CHCl_3$ -alcohol solvent (90+10), using 30, 20, 20, 10, 10, and 5 ml, or until the alkaloid is completely removed. Test for complete extraction of alkaloid. (Make an additional extraction with 10 ml of the  $CHCl_3$ -alcohol solvent, evaporate solvent in separate beaker, dissolve residue in a few drops of methyl alcohol, add one drop of methyl red, 39.8(b), and dilute with 20 ml of  $H_2O$ , carbonate-free. Yellow color indicates incomplete extraction. Titrate, and add quantity thus obtained to total.) Combine  $CHCl_3$ -alcohol extracts in a second separator, into stem of which is inserted pledget of cotton wet with  $CHCl_3$ . Wash combined extracts with 1 ml of  $H_2O$ . When clear, filter into small beaker. Extract wash  $H_2O$  twice with small portions of the  $CHCl_3$ -alcohol solvent. Evaporate on water bath, using electric fan to prevent decrepitation of residue. When dry, remove immediately and complete determination by one of following procedures:

(a) To alkaloidal residue add 2-3 ml of methyl alcohol, cover beaker with watch-glass, and heat on steam bath until residue, including any portions thereof that may adhere to upper part of beaker, is completely dissolved. Add 2 drops of the methyl red indicator and, without dilution with  $H_2O$ , titrate carefully with 0.02  $N$   $H_2SO_4$  to faint pink, avoiding excess. Cover beaker and digest on steam bath until all particles are completely dissolved. If more than 2 ml of alcohol is added, evaporate excess. Cool, and dilute with 50 ml of boiled  $H_2O$ . (Soln should now be yellow.) Finish titration with the standard acid to faint red.

(b) Dissolve residue in 2-3 ml of methyl alcohol on steam bath. Add 2 drops of the methyl red indicator and then add from buret 5-10 ml excess of 0.02  $N$   $H_2SO_4$ , noting total quantity used. Cover beaker with watch-glass and heat on steam bath until residue, including any portions thereof that may adhere to upper part of beaker, is completely dissolved. Dilute with 50 ml of cold, previously boiled  $H_2O$ . Titrate back with the 0.02  $N$  NaOH. The  $H_2O$  and alkali should be sufficiently free from carbonates to insure a sharp end point with methyl red. 1 ml of 0.02  $N$  acid = 0.00751 g of morphine hydrochloride,  $C_{17}H_{19}O_2N \cdot HCl \cdot 3H_2O$ , or 0.0076 g of morphine sulfate,  $(C_{17}H_{19}O_2 \cdot N)_2 \cdot H_2SO_4 \cdot 5H_2O$ .

Alkaloids other than morphine are extracted by  $CHCl_3$ , while morphine remains in the fixed alkali soln. In general, this separation is unnecessary. If tablets are of unknown composition or atropine or scopolamine is present, shake the alkaline NaCl soln with 10 ml portions of washed  $CHCl_3$  (use ether for separation of atropine). Transfer clear solvent to small beaker and evaporate on steam bath. If a residue is obtained, apply usual tests.

## 39.97

## MORPHINE IN SIRUPS (47)—OFFICIAL

Shake bottle well and transfer 50 ml to 150 ml pear-shaped separator. Add a few drops of  $\text{NH}_4\text{OH}$  (10%  $\text{NH}_3$ ) to insure a weak alkaline reaction and test with litmus paper. Extract total alkaloids with  $\text{CHCl}_3$ -alcohol (9+1). (About seven 25 ml portions are necessary; number required depends on care used in separating solvent and length and violence of each shake-out. Larger quantities of solvent may be used to insure absence of emulsions.) Combine solvents and wash with 5 ml of  $\text{H}_2\text{O}$ . Run thru  $\text{CHCl}_3$ -wetted cotton. Evaporate solvent. (If sirup is known to carry pure morphine alkaloid or its salt, and no other alkaloid, this residue may be dissolved in alcohol and filtered, and the soln titrated.) Dissolve in 2 ml of 5%  $\text{HCl}$  on water bath, covering beaker to insure complete soln. Add 20 ml of  $\text{H}_2\text{O}$  and transfer to separator. Make alkaline with 5 ml of 5%  $\text{KOH}$  soln and exhaust with three 20 ml portions of  $\text{CHCl}_3$  followed by two 20 ml portions of petroleum benzine (removal of non-phenolic alkaloids and  $\text{CHCl}_3$ ). Combine immiscible solvents and wash with 5 ml of  $\text{H}_2\text{O}$ . Discard solvent and add wash  $\text{H}_2\text{O}$  to main aqueous soln.

Render aqueous soln acid with 5%  $\text{HCl}$  and then just alkaline with a few drops of 10%  $\text{NH}_3$  and extract with three 20 ml portions of petroleum benzine for removal of petroleum benzine-soluble phenolic alkaloids. Wash combined petroleum benzine extract with 5 ml of  $\text{H}_2\text{O}$ . Discard petroleum benzine and add wash  $\text{H}_2\text{O}$  to main aqueous soln. Saturate with  $\text{NaCl}$ .

Extract the morphine completely with seven 25 ml portions of the  $\text{CHCl}_3$ -alcohol mixture. Combine portions of solvent. Wash with 5 ml of  $\text{H}_2\text{O}$  and run solvent thru plug of  $\text{CHCl}_3$ -saturated cotton. Evaporate solvent. Dissolve residue in 5 ml of neutralized alcohol in covered beaker by aid of heat on steam bath. Add excess of 0.02  $N$   $\text{H}_2\text{SO}_4$  and titrate back with 0.02  $N$  alkali, using methyl red indicator. 1 ml of 0.02  $N$   $\text{H}_2\text{SO}_4$  = 0.0076 g of morphine sulfate,  $(\text{C}_{17}\text{H}_{19}\text{NO})_2 \cdot \text{H}_2\text{SO}_4 \cdot 5\text{H}_2\text{O}$ .

## PHYSOSTIGMINE SALICYLATE IN TABLETS (48)—TENTATIVE

## 39.98

## PREPARATION OF SAMPLE—See 39.1

## 39.99

## DETERMINATION

Weigh accurately sufficient quantity of powdered material to contain ca 0.065 g of physostigmine salicylate, transfer to separator, and add sufficient  $\text{H}_2\text{O}$  (not exceeding 20 ml) to dissolve. Make alkaline to litmus with solid  $\text{NaHCO}_3$ , and extract at once with  $\text{CHCl}_3$ , using 30, 20, 10, and 10 ml portions. Transfer each extract to second separator containing 5 ml of  $\text{H}_2\text{O}$ . Wash each extract with this  $\text{H}_2\text{O}$  and filter solvent into beaker, using cotton pledget moistened with  $\text{CHCl}_3$ . Test for complete extraction by making additional extraction with 5 ml portion of  $\text{CHCl}_3$  and treating separately as directed below. Evaporate combined  $\text{CHCl}_3$  extracts on water bath to ca 5 ml, using current of air to assist evaporation, remove beaker from bath, and complete evaporation without aid of heat. Dissolve residue in a few ml of neutral alcohol. Add excess of 0.02  $N$   $\text{H}_2\text{SO}_4$ . Cover with watch-glass and heat on steam bath until alkaloids have been washed down sides by refluxing action. Remove watch-glass and evaporate bulk of alcohol. Cool. Add methyl red indicator, 39.8(b), and titrate excess acid with 0.02  $N$   $\text{NaOH}$ . 1 ml of 0.02  $N$   $\text{H}_2\text{SO}_4$  = 0.00827 g of physostigmine salicylate,  $\text{C}_{15}\text{H}_{21}\text{O}_2\text{N}_3 \cdot \text{C}_7\text{H}_5\text{O}_2$ .

## 39.100

## PILOCARPINE HYDROCHLORIDE IN TABLETS (49)—OFFICIAL

Ascertain average weight per tablet. Pulverize, mix thoroly, and weigh out a sufficient portion to represent ca 0.065 g of the salt. Dissolve sample in 10 ml of  $\text{H}_2\text{O}$ ,

add 1 ml of 10%  $\text{NH}_4\text{OH}$ , and shake out rapidly with 20 ml of  $\text{CHCl}_3$ . Repeat extraction, using 15 ml of  $\text{CHCl}_3$ , and complete with successive 10 ml portions. Filter each portion of  $\text{CHCl}_3$  thru pledget of cotton and combine in 250 ml beaker, finally washing stem of separator and funnel with  $\text{CHCl}_3$ . Evaporate on steam bath until  $\text{CHCl}_3$  soln measures ca 5 ml. Add 20 ml of 0.02  $N$   $\text{H}_2\text{SO}_4$  and evaporate remainder of  $\text{CHCl}_3$ . Titrate excess acid with 0.02  $N$   $\text{NaOH}$ , using 1 drop of methyl red as indicator. (End point is not particularly sharp, but with care it can be obtained.) 1 ml of 0.02  $N$   $\text{H}_2\text{SO}_4$  = 0.00489 g of pilocarpine hydrochloride,  $\text{C}_{11}\text{H}_{16}\text{O}_2\text{N}_2 \cdot \text{HCl}$ .

#### PROCAINE

##### 39.101

##### *Qualitative Tests (50)—Official*

(a) Dissolve 0.1 g of sample in ca 10 ml of  $\text{H}_2\text{O}$ . Add 2 ml of 5%  $\text{KMnO}_4$  soln. Warm, if necessary. Reduction occurs with evolution of gas having odor of acetaldehyde (distinction from cocaine, which does not readily reduce  $\text{KMnO}_4$ ).

(b) Dissolve ca 0.005 g of sample in 3 ml of  $\text{H}_2\text{O}$  and add a few drops of Mayer reagent, 39.76. With procaine a white precipitate is formed, which dissolves after addition of a few ml of  $\text{H}_2\text{SO}_4$  (1+49). (Precipitates with stovaine and cocaine are not readily soluble in dilute  $\text{H}_2\text{SO}_4$ .)

(c) Dissolve ca 0.1 g of procaine in 2 ml of  $\text{H}_2\text{O}$ . From a buret add 25 ml of 0.1  $N$   $\text{NaOH}$ . (White precipitate formed dissolves in excess of the  $\text{NaOH}$  when heated.) Heat soln 25 min. on steam bath. Upon cooling the soln, extracting with  $\text{CHCl}_3$ , and evaporating solvent, no residue should be obtained. Stovaine does not readily hydrolyze, and a residue giving an alkaloidal reaction remains upon evaporation of the  $\text{CHCl}_3$ .

##### *Quantitative Methods*

##### 39.102

##### *Method I—Official*

(Determines as procaine any  $p$ -amino-benzoic acid formed by decomposition)

Dissolve quantity of sample equivalent to ca 0.1 g of procaine hydrochloride in 5 ml of  $\text{H}_2\text{O}$  in 50 ml beaker. Add 25 ml of 0.1  $N$   $\text{NaOH}$  and heat on steam bath 25 min. Cool, and transfer soln to 500 ml glass-stoppered flask. Add 50 ml of standard bromide-bromate soln, 39.28(c), dilute with  $\text{H}_2\text{O}$  to 250 ml, add 10 ml of  $\text{HCl}$ , and stopper flask immediately to avoid loss of  $\text{Br}$ . Shake flask occasionally and allow to stand 2 hours at room temp., keeping flask tightly stoppered. (It is necessary that a large excess of  $\text{Br}$  shown by a bright yellow color be present.) Add quickly 10 ml of 20%  $\text{KI}$  soln, stopper, and shake flask. Allow to stand 15 min., shaking occasionally. Titrate excess  $\text{I}$  with 0.1  $N$   $\text{Na}_2\text{S}_2\text{O}_3$ , using starch indicator, 6.3(e). Titrate to disappearance of the blue color, disregarding color that reappears on standing. 1 ml of 0.1  $N$  bromide-bromate = 0.00454 g of procaine hydrochloride,  $\text{C}_{13}\text{H}_{20}\text{O}_2\text{N}_2 \cdot \text{HCl}$ .

##### 39.103

##### *Method II—Official*

(Determines only undecomposed procaine)

Weigh quantity of powder or number of tablets equivalent to ca 0.2 g of procaine. Dissolve in 10–15 ml of  $\text{H}_2\text{O}$ , transfer soln to a separator, and add ca 3 ml of  $\text{NH}_4\text{OH}$ . Extract the ammoniacal soln 4 or 5 times with  $\text{CHCl}_3$ , using 15 ml for first extraction and 10 ml for subsequent extractions. Filter into weighed beaker and evaporate the  $\text{CHCl}_3$  by means of electric fan, preferably at room temp., avoiding prolonged heating of procaine base, as it is slightly volatile at  $100^\circ$ . Take up residue with slight

excess of 0.1 *N* or 0.02 *N*  $\text{H}_2\text{SO}_4$ . Titrate excess of acid with 0.02 *N*  $\text{NaOH}$ , using methyl red indicator. 1 ml of 0.1 *N*  $\text{H}_2\text{SO}_4 = 0.0273$  g of  $\text{C}_{11}\text{H}_{20}\text{O}_2\text{N}_2 \cdot \text{HCl}$ . 1 ml of 0.02 *N*  $\text{H}_2\text{SO}_4 = 0.00546$  g of  $\text{C}_{11}\text{H}_{20}\text{O}_2\text{N}_2 \cdot \text{HCl}$ .

## 39.104

*Method III (51)—Official*

(Applicable in presence of chlorobutanol, cocaine, codeine, heroine, lactose, and morphine)

Weigh into Kjeldahl flask 0.3–0.5 g of procaine or one of its salts, or measure an equivalent of an ampul soln. Dissolve in 150 ml of  $\text{H}_2\text{O}$  (or add sufficient  $\text{H}_2\text{O}$  to make 150 ml), and add 2 ml of 50%  $\text{NaOH}$ . Quickly connect to condenser and distil 100 ml into flask containing measured excess of standard acid, extending delivery tube below surface of the soln. Remove receiver, rinse condenser with a little  $\text{H}_2\text{O}$ , and titrate excess acid with standard alkali, using methyl red as indicator. Each ml of 0.1 *N* acid consumed = 0.0236 g of procaine,  $\text{C}_{11}\text{H}_{20}\text{O}_2\text{N}_2$ , or 0.0273 g of procaine hydrochloride,  $\text{C}_{11}\text{H}_{20}\text{O}_2\text{N}_2 \cdot \text{HCl}$ .

## 39.105

## STRYCHNINE IN TABLETS (52)—OFFICIAL

(Other alkaloids absent)

Count and weigh sufficient tablets (or pills) to represent 1 grain of the alkaloidal salt and transfer to small beaker. If color on coated tablets interferes with the indicator in titration, wash off without removing the strychnine. Add 10 ml of 5%  $\text{HCl}$ , disintegrate tablets with stirring rod, warm on steam bath ca 10 min., cool, and transfer to separator with not more than 10 ml of  $\text{H}_2\text{O}$ . To remove all the strychnine, add to beaker 2 ml of  $\text{NH}_4\text{OH}$  (or an excess) and 25 ml of  $\text{CHCl}_3$ , rinse, and add to separator. Then rinse beaker with portions of  $\text{CHCl}_3$  to be used for each extraction. Extract 5 times with  $\text{CHCl}_3$ , using 25, 20, 15, 10, and 5 ml portions, or until alkaloid is completely removed. Combine first two extractions in a second separator, in stem of which is a pledget of absorbent cotton wet with  $\text{CHCl}_3$ . Wash with 5 ml of  $\text{H}_2\text{O}$  containing a drop of  $\text{NH}_4\text{OH}$  (1 + 2). When clear, filter  $\text{CHCl}_3$  into small beaker. Wash each successive  $\text{CHCl}_3$  extract with same wash  $\text{H}_2\text{O}$  and filter in similar manner into main portion, finally washing outer surface of stem of separator with a few ml of  $\text{CHCl}_3$  and adding this also to main portion. Evaporate on steam bath, removing dish from bath as last portions evaporate to avoid decrepitation.

Add 2–5 ml of neutral alcohol, cover beaker, and warm on steam bath to dissolve residue. If necessary, add just enough additional neutral alcohol to complete soln. Add 2 drops of methyl red indicator, and titrate with 0.02 *N*  $\text{H}_2\text{SO}_4$  to faint pink color. If more than 2 ml of alcohol was used, evaporate excess, cool, dilute with 50 ml of recently boiled  $\text{H}_2\text{O}$ , and continue titration with the 0.02 *N*  $\text{H}_2\text{SO}_4$  to faint pink color. If preferred, add an excess of 0.02 *N*  $\text{H}_2\text{SO}_4$  to alcoholic soln of the alkaloids, evaporate the alcohol if necessary as directed above, and titrate excess acid with 0.02 *N*  $\text{NaOH}$ .

1 ml of 0.02 *N*  $\text{H}_2\text{SO}_4 = 0.00669$  g of  $\text{C}_{21}\text{H}_{22}\text{O}_2\text{N}_2$ , 0.00857 g of  $(\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_2)_2 \cdot \text{H}_2\text{SO}_4 \cdot 5\text{H}_2\text{O}$ , or 0.00795 g of  $\text{C}_{21}\text{H}_{22}\text{O}_2\text{N}_2 \cdot \text{HNO}_3$ .

## 39.106

## STRYCHNINE IN LIQUID PREPARATIONS (53)—OFFICIAL

(Other alkaloids absent)

Measure into evaporating dish 50 ml of sample, or quantity sufficient to yield at least 0.065 g of the alkaloid, and remove alcohol by evaporation. Transfer to separator, add 1 ml of  $\text{NH}_4\text{OH}$ , or sufficient to render soln alkaline, and proceed as directed under 39.105, beginning "Extract 5 times with  $\text{CHCl}_3$ ."

## SEPARATION OF QUININE AND STRYCHNINE (54)—TENTATIVE

## 39.107

## TOTAL ALKALOIDS

Make 50 ml of the soln acid with citric acid, add equal volume of  $H_2O$ , evaporate to nearly original volume to remove excess alcohol, cool, and extract with two 15 ml portions of ether to remove oily material. Make aqueous soln alkaline with  $NH_4OH$  and extract mixed alkaloids in usual way with mixture of 2 parts of  $CHCl_3$  and 1 part of ether, using 25, 20, 15, 10, and 5 ml portions. Evaporate the  $CHCl_3$  and ether in weighed Erlenmeyer flask or beaker to dryness on steam bath. Add a little ether and again evaporate to dryness to remove last traces of  $CHCl_3$ . Dry at  $100^\circ$  for 1 hour and weigh to obtain approximate weight of mixed alkaloids.

(a) *Strychnine*.—Dissolve alkaloidal residue in 50 ml of 10%  $H_2SO_4$ , add 5 ml of 4%  $K_4Fe(CN)_6 \cdot 3H_2O$  dropwise from buret, stirring well, and set aside a few hours, or overnight. Collect resulting precipitate on small (7 cm) filter and wash 3 times with 3 ml of 5%  $H_2SO_4$ . Reserve filtrate for determination of quinine. Wash precipitate immediately into small separator with  $H_2O$ , transferring precipitate remaining in flask to separator by shaking about 3 times with 3 ml of  $NH_4OH$  and a small quantity of  $CHCl_3$ . Extract ammoniacal soln of precipitate with 25, 15, 15, 10, and 5 ml portions of  $CHCl_3$ . Collect the  $CHCl_3$  solns in another separator and extract the alkaloids by shaking with 25, 10, 10, and 5 ml portions of 20%  $H_2SO_4$ ; repeat precipitation with  $K_4Fe(CN)_6$  and the other operations until the  $CHCl_3$  extracts are again obtained, reserving filtrate for determination of quinine. Evaporate the  $CHCl_3$  carefully, adding a little alcohol toward end to prevent spattering. Weigh residue of strychnine after drying it for 1 hour at  $100^\circ$  (should be nearly white and free from quinine). Check volumetrically as follows: Dissolve residue in hot alcohol, add 0.02  $N$   $H_2SO_4$  until soln is acid to methyl red indicator, 39.8(b), then add 2 or 3 ml in excess. Evaporate most of the alcohol, cool, and titrate back with 0.02  $N$  alkali. 1 ml of 0.02  $N$  acid = 0.00669 g of strychnine,  $C_{21}H_{22}O_2N_2$ , or 0.00857 g of strychnine sulfate,  $(C_{21}H_{22}O_2N_2)_2 \cdot H_2SO_4 \cdot 5H_2O$ .

(b) *Quinine*.—Combine the 2 filtrates from the precipitations with  $K_4Fe(CN)_6$  in separator, make alkaline with  $NH_4OH$ , and extract with mixture of 2 parts of  $CHCl_3$  and 1 part of ether, using 20, 15, 15, 10, and 5 ml portions of the solvent and observing the usual precaution of washing stem of separator with the  $CHCl_3$ -ether mixture. Wash combined extractions in second separator with two 5 ml portions of  $H_2O$ , transfer to weighed beaker, evaporate to dryness, add a few ml of ether, and again evaporate to dryness to remove final traces of  $CHCl_3$ . Dry at  $120$ – $130^\circ$ , cool, and weigh as anhydrous quinine. Test residue qualitatively for quinine, or if desired, check the quantity volumetrically as follows: Dissolve residue in a little alcohol, add 7 drops of the bromocresol purple indicator, 39.11, then add 0.02  $N$   $H_2SO_4$  to a yellow color, and 1 ml in excess. Evaporate the soln to small volume, cool, allow the quinine sulfate to separate, filter thru small pledget of cotton in stem of a funnel, wash with small portions of  $H_2O$ , and titrate combined filtrate and washings with 0.02  $N$  alkali. 1 ml of 0.02  $N$   $H_2SO_4$  = 0.00649 g of anhydrous quinine,  $C_{20}H_{24}O_2N_2$ ; 0.00757 g of quinine alkaloid,  $C_{20}H_{24}O_2N_2 \cdot 3H_2O$ ; or 0.00783 g of quinine sulfate,  $(C_{20}H_{24}O_2N_2)_2 \cdot H_2SO_4 \cdot 2H_2O$ .

## THEOBROMINE IN THEOBROMINE CALCIUM (55)

## 39.108

*Method I—Official, First Action*

Dry ca 0.5 g of the material at  $110^\circ$  to constant weight. Weigh 0.2 g of the dried substance into glass-stoppered 100 ml volumetric flask, add 2 ml of acetic acid,



and warm on steam bath. Add 10 ml of boiling  $H_2O$  and shake until soln has taken place, adding more boiling  $H_2O$  if necessary. Cool soln to room temp. (Soln should be clear or nearly so.) Add 50 ml of 0.1 *N*, 20 ml of saturated NaCl soln, and 2 ml of HCl. Shake well and make to volume with  $H_2O$ . Shake again and allow to stand overnight. Filter, discarding first 10 ml of filtrate. Titrate 50 ml of filtrate with 0.1 *N*  $Na_2S_2O_3$ , using starch soln, 6.3(e), as indicator. 1 ml of 0.1 *N* I = 0.00451 g of theobromine,  $C_7H_5O_2N_4$ .

*Method II—In Tablets (56)—Official, First Action*

39.109

## INDICATOR

*Phenol red soln.*—Triturate 0.1 g of phenol red in agate mortar with 15 ml of 0.02 *N* NaOH until dissolved and dilute soln with recently boiled  $H_2O$  to 200 ml.

39.110

## DETERMINATION

Place 0.5 g of the powdered tablets, previously dried at  $110^\circ$ , or 0.4 g of theocalcin powder, or 0.2 g of theobromine alkaloid, in 300 ml beaker and add 100 ml of  $H_2O$ . Warm moderately over a flame and add 15 ml of ca 0.1 *N*  $H_2SO_4$ . Heat to boiling to insure complete soln and to remove  $CO_2$ . Cool to room temp. Add 1.5 ml of the phenol red indicator and render slightly alkaline with ca 0.1 *N* NaOH (red-violet color), then titrate carefully to an acid reaction with 0.1 *N*  $H_2SO_4$  (yellow color). To this soln add 25 ml (an excess) of neutral 0.1 *N*  $AgNO_3$ , 6.91, and titrate the liberated  $HNO_3$  immediately with 0.1 *N* NaOH to a distinctly violet red color. Titrate cautiously dropwise with constant stirring near end point. 1 ml of 0.1 *N* NaOH = 0.01802 g of  $C_7H_5O_2N_4$ .

39.111

## THEOPHYLLINE (57)—OFFICIAL

(Applicable to solutions and tablets)

Weigh 0.2–0.3 g of theophylline (or an equivalent quantity of soln or powdered tablets) into separator. Add 5 ml of 0.5 *N* NaOH and shake mixture gently until alkaloid is dissolved. Add a strip of litmus paper and sufficient 0.5 *N* HCl from buret to produce a distinct acid reaction. Then add 0.5 ml more of the acid. Add 30 ml of  $CHCl_3$ -isopropyl alcohol mixture (3+1) and shake 1 min. Allow to settle and draw off lower layer into second separator that contains 10 ml of  $H_2O$  acidified with HCl. Shake well, allow to settle, and filter solvent into weighed flask thru pledget of cotton placed in stem of a funnel. Repeat extraction with 6 more portions of 20 ml each of the  $CHCl_3$ -isopropyl alcohol mixture, wash each portion thru the second separator, and pass solvent thru filter into the weighed flask. Insure complete extraction by a seventh shaking with 10 ml of the solvent and evaporation of the washed solvent in separate container. Recover most of solvent and evaporate remainder on steam bath while rotating container in inclined position. Add 2 ml of absolute ether to residue and evaporate (cautiously to avoid spattering). Dry residue at  $80^\circ$  to constant weight and weigh as anhydrous theophylline.  $C_7H_8O_2N_4 \times 1.10 = C_7H_5O_2N_4 \cdot H_2O$ .

39.112

## ALOIN (58)—OFFICIAL

(Applicable to mixtures containing cascara, rhubarb, senna, and other acid hydrolyzable anthraglucosides, as well as to resins and phenolphthalein with aloin. Not applicable to aloes.)

Dry sufficient powdered material 1 hour at  $110^\circ$  (or dealcoholized soln if a liquid) to insure ca 0.3 g of aloin. Add 10 ml of  $H_2O$  and a few ml of 5% NaOH soln. Trans-

fer mixture to a 100 ml volumetric flask, dilute to ca 75 ml, and make acid with  $\text{H}_2\text{SO}_4$ , working rapidly as aloin is attacked by alkali. Dilute to mark, and add a few glass beads if much undissolved material is present. Shake occasionally during an hour to insure soln of aloin. Filter, and transfer a 40 ml aliquot, to which has been added 10 ml of 10%  $\text{H}_2\text{SO}_4$  (by weight), to a continuous extraction apparatus previously charged with  $\text{CHCl}_3$  (A, Fig. 65). Reflux to exhaustion (ca 2 hours). Disconnect apparatus and transfer all aqueous soln to a separator, discarding the  $\text{CHCl}_3$ . Saturate soln with  $\text{NaCl}$  and shake out with 30 ml portions of  $\text{CHCl}_3$ -alcohol mixture (3+1). Test for complete removal of aloin by evaporating a portion of the 6th extraction (more extractions may be necessary). Shake violently. Combine extracts and wash with 1 ml of  $\text{H}_2\text{O}$ , to which has been added 1 g of  $\text{NaHCO}_3$ , or more if necessary to insure an excess. Filter, evaporate, add 5 ml of  $\text{CHCl}_3$ , evaporate, dry at  $110^\circ$  for 1 hour, cool, and weigh rapidly. Weight = aloin in aliquot taken. As a check, acetylate the aloin. This may be done by dissolving in acetic anhydride (ca 10 ml), adding excess (ca 2 g) of powdered anhydrous Na acetate and boiling 5 min. in an acetylation flask placed in oil bath. Wash sample from flask with additional acetic anhydride and evaporate to apparent dryness in hood with good draft. Add 10 ml of  $\text{H}_2\text{O}$  and heat several minutes. Transfer with aid of  $\text{CHCl}_3$  to separator, washing flask with successive portions of  $\text{CHCl}_3$ , and shake out with two additional 10 ml portions of  $\text{CHCl}_3$  (the aloin hexaacetate formed is soluble in  $\text{CHCl}_3$ ). Combine the  $\text{CHCl}_3$  portions, filter, evaporate, add 10 ml of  $\text{CHCl}_3$ , evaporate, dry at  $110^\circ$  for 1 hour, cool, and weigh. Weight  $\times 0.615$  = aloin.

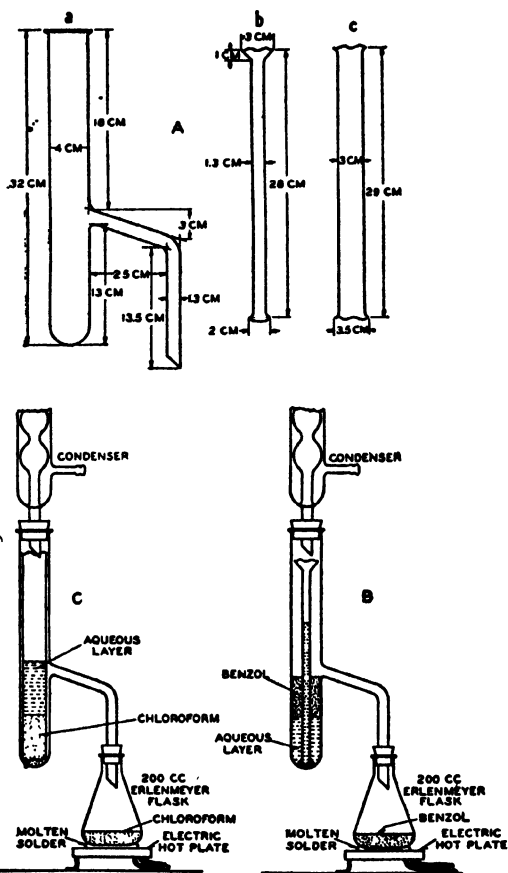


FIG. 65.—CONTINUOUS EXTRACTION APPARATUS

## CASCARA SAGRADA (59)—TENTATIVE

## 39.113

## REAGENT

*Sodium bicarbonate soln.*—(5+100). Make up in cold  $\text{H}_2\text{O}$  as needed; add 1 ml of 0.1 N HCl to insure freedom from  $\text{Na}_2\text{CO}_3$ .

## 39.114

## DETERMINATION

Introduce  $\text{CHCl}_3$  into the continuous extraction apparatus (A, Fig. 65) to within 5 cm of the overflow. Adjust a 200 ml Erlenmeyer flask carrying 125 ml of  $\text{CHCl}_3$  to the apparatus with well-fitted, tin-foiled cork. Into inner tube of apparatus introduce a measured or weighed portion of sample representing ca 2 g of cascara sagrada. Add 20 ml of  $\text{H}_2\text{O}$  and 1 ml of acetic acid (1+100) to cascara layer. Connect apparatus to condenser. (Outlet of condenser should not be constricted. If it is, place a hole in side near its tip to insure free return of  $\text{CHCl}_3$ .) Adjust burner, using asbestos ring to prevent overheating, and reflux rapidly 2 hours. ( $\text{CHCl}_3$  in tube will be colorless.) Disconnect flask and discard its contents. Recharge the Erlenmeyer flask with 125 ml of  $\text{CHCl}_3$  and connect to apparatus, which still carries the  $\text{CHCl}_3$ -exhausted acetic acid soln of original sample and the clear exhausted  $\text{CHCl}_3$ . Add 10 ml of  $\text{H}_2\text{SO}_4$  (1+1) to the cascara layer by means of a pipet.

Connect apparatus to condenser, adjust burner, and reflux rapidly. At end of 3 hours  $\text{CHCl}_3$  in apparatus should be practically colorless, but it may contain a small quantity of color, a non-emodin material. Remove flame and disconnect flask. Transfer the  $\text{CHCl}_3$  in flask to a separator, wash flask with 10 ml of  $\text{H}_2\text{O}$ , and transfer the  $\text{H}_2\text{O}$  to the separator carrying  $\text{CHCl}_3$ . Shake, withdraw the  $\text{CHCl}_3$ , and again wash the  $\text{H}_2\text{O}$  with 10 ml of  $\text{CHCl}_3$ , adding washings to main  $\text{CHCl}_3$  soln. Wash the  $\text{CHCl}_3$  with three 10 ml portions of the  $\text{NaHCO}_3$  soln, then wash the combined reagent with  $\text{CHCl}_3$  two or three times. Discard the aqueous soln.

Shake out the combined  $\text{CHCl}_3$  to exhaustion with saturated  $\text{Na}_2\text{CO}_3$  soln in a train of separators (four 10 ml portions should suffice). Wash the combined reagent with  $\text{CHCl}_3$  several times. Discard all the  $\text{CHCl}_3$ .

Add sufficient  $\text{HCl}$  (1+1) to the aqueous soln (cautiously, a few ml at a time) to insure an acid reaction. Extract with  $\text{CHCl}_3$  in a separator or automatic extractor to completion. Combine the  $\text{CHCl}_3$  extracts, wash with 5 ml of  $\text{H}_2\text{O}$ , and filter thru filter wetted with  $\text{CHCl}_3$ . Evaporate to 20 ml. Transfer residue to small glass or Pt dish, evaporate to dryness, and dry at  $100^\circ$  for 2 hours. Cool, and weigh as the hydrolyzed products from the anthraglucosides of cascara.

## 39.115

## TOTAL ALKALOIDS IN EPHEDRA (60)—OFFICIAL

Place 10 g of ephedra, in fine powder, in Erlenmeyer flask. Add exactly 100 ml of solvent consisting of 3 volumes of ether and 1 volume of  $\text{CHCl}_3$  (cooled to working temp. after mixing). Stopper securely, shake, and allow to stand at least 5 min. Add 5 ml of  $\text{NH}_4\text{OH}$  soln (10%  $\text{NH}_3$ ) and 0.5 g of anhydrous  $\text{Na}_2\text{CO}_3$ , stopper tightly, and macerate at least 4 hours, with occasional shaking. Decant or filter rapidly a 50 ml aliquot of the clear supernatant liquid representing 5 g of the drug, transfer to separator, and shake with 3 portions of 2%  $\text{H}_2\text{SO}_4$ , using 15, 10, 10 ml, etc., until extraction is complete. Combine the acid solns in a separator, neutralize with  $\text{NH}_4\text{OH}$ , and add ca 5 g of anhydrous  $\text{Na}_2\text{CO}_3$ , stirring until dissolved. Shake with 5 portions of ether, using 35, 30, 25, 20, and 15 ml, until extraction is complete, and combine the ether portions in second separator. When clear, decant, and filter into small beaker thru pledget of cotton previously wet with ether.

Evaporate ether to ca 10 ml on steam bath with moderate heat. Add bromothymol blue indicator, 6.117(e), a measured excess of 0.02 N  $\text{H}_2\text{SO}_4$ , and ca 40 ml of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ . Cover with watch-glass, return to steam bath in order to dissolve any alkaloid adhering to sides of beaker, and then evaporate the ether. Titrate excess acid with 0.02 N alkali. 1 ml of 0.02 N acid = 0.00330 g of ephedra alkaloids.

## IDENTIFICATION OF GUMS (61)—TENTATIVE

39.116

## REAGENTS

(a) *Chlorzinciodide soln.*—To 100 ml of a soln of  $\text{ZnCl}_2$ , sp. gr. 1.8, add a soln of 10 g of KI and 0.15 g of I in 10 ml of  $\text{H}_2\text{O}$ . Keep a few crystals of I in the soln.

(b) *Ruthenium red soln.*—To a few ml of 10% Pb acetate soln add enough ruthenium red [ $\text{Ru}_2(\text{OH})_2\text{Cl}_4 \cdot 7\text{NH}_3 \cdot 3\text{H}_2\text{O}$ ] to produce a wine red color.

(c) *Alcoholic methylene blue soln.*—0.1% soln in alcohol.

(d) *Aqueous methylene blue soln.*—0.1% soln in  $\text{H}_2\text{O}$ .

39.117

## PREPARATION OF SAMPLES

(a) *Controls.*—Moisten 1 g of the dry gum with alcohol, add 100 ml of  $\text{H}_2\text{O}$  with constant stirring, and bring to boil. To 5 or 10 ml of resulting liquid or jelly, add 4 volumes of alcohol, mix, and centrifuge to bring precipitate together as compact mass. (Some gums, notably acacia and agar, may fail to be thrown down by this treatment. Addition of a few drops of saturated NaCl soln should cause rapid flocculation and settling.)

(b) *Jellies or lotions.*—Stir, and add  $\text{H}_2\text{O}$  if necessary to produce a fluid mass. Treat portion of sample with alcohol to precipitate the gum as directed under (a). Remove fatty or oily material, if present, by washing precipitated gum with ether, then redissolve in  $\text{H}_2\text{O}$  and re-precipitate.

39.118

## DETERMINATION

With a clean towel squeeze a small lump of the alcohol precipitate against a slide to form a mat 4–8 mm in diam. on the slide. Note character of resulting mat as possible index to type of gum. Quince and Irish moss form thin and rather translucent films, while agar, starch, and acacia are white and opaque. Cover mat with a large drop of the chlorzinciodide soln and observe carefully both with and without magnification. For direct examination place slide upon white surface. For microscopic examination use a magnification of ca 90 diameters. If no characteristic color is produced within 1–2 min., proceed with a fresh mat to examine for the group, 39.119. Continue in similar manner thru all group tests or until identity is established. Use fresh mat for each individual test.

39.119

*Characteristics of tests for gums*

## Group I.—Reagent, Chlorzinciodide

GUM	ORIGINAL ALCOHOL PPT.	GROUP REACTION	CONFIRMATORY TEST	REMARKS
Tragacanth	Stringy Bluish Translucent	Blue	Warm with 10% NaOH on steam bath Yellow	Certain gums, e.g., Irish moss, may yield dull yellow color with NaOH. Tragacanth bright yellow
Starch	White Compact	Blue black	Iodine, 0.1 N Blue	Tragacanth may yield faint blue
Quince	Stringy Translucent	Blue	Above tests negative	Quince is distinguished from starch and tragacanth by negative reactions
Irish moss	Stringy	Brown (small blue particles)	Characteristic nodular structures with group reagent	Old preparations of this gum may fail to show characteristic structures

Group II.—Reagent, Tincture of Iodine U.S.P.  
(Allow tincture to dry on mat, flush off with alcohol, and irrigate with H<sub>2</sub>O.)

Agar	White opaque	Opaque blue black	Stains with ruthenium red	Does not dissolve or lose shape when covered with H <sub>2</sub> O
Irish moss	Stringy	Brown or lilac	Characteristic blue stain with alcoholic methylene blue	These reactions yielded by old as well as fresh preparations

Group III.—Reagent, Ruthenium Red

Karaya	Fine flocculent compact mass on centrifuging	Swells considerably. Strongly stained pink granular mass	Heat with HCl. Pink	Aqueous methylene blue produces a characteristic blue stain
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Group IV.—Reagent, H<sub>2</sub>SO<sub>4</sub>  
(Warm cautiously on steam bath)

Galagum	Stringy	Pink or red brown	No satisfactory test found	Alcohol precipitate from galagum resembles that from tragacanth
Acacia		Greenish brown	Ppt. completely soluble in H <sub>2</sub> O	Complete soln of acacia distinguishes it from most other gums

#### TOTAL ALKALOIDS IN IPECAC, FLUIDEXTRACT

##### 39.120

##### PREPARATION OF SOLUTION

Pipet 20 ml of the fluidextract into 100 ml volumetric flask, add ca 5 ml of normal H<sub>2</sub>SO<sub>4</sub>, and with aid of air blast evaporate on steam bath to volume of ca 10 ml. Then, while rotating flask, add ca 30 ml of H<sub>2</sub>O, cool to room temp., and make to mark with H<sub>2</sub>O. Allow to stand overnight and filter thru dry filter, rejecting first few ml of filtrate.

##### DETERMINATION

##### 39.121

##### *Automatic Extraction Method (62)—Tentative*

Measure 20 ml of prepared filtrate (4 ml of fluidextract of ipecac) into automatic extractor (B, Fig. 65), that has been fitted to 200 ml Erlenmeyer flask. Add 60 ml of H<sub>2</sub>O, 2 ml of 8% NH<sub>4</sub>OH soln, and ca 50 ml of peroxide-free ether. Shake gently to prevent deposition of any solid matter on bottom of extractor and then add peroxide-free ether until ca 75 ml has passed over into flask. Heat flask on steam bath (not electric hot plate) and extract 2 hours, or until extraction is complete. Separate ether from aqueous layer and add it to main concentrate in flask. Evaporate combined ether extracts on steam bath, add 2–3 ml of absolute alcohol, and repeat evaporation to remove all traces of NH<sub>3</sub>. Warm alkaloidal residue on steam bath with 2–3 ml of neutral alcohol to insure complete soln. Add 10 ml of 0.1 N

H<sub>2</sub>SO<sub>4</sub>, and dilute with ca 20 ml of recently boiled, cooled H<sub>2</sub>O. Titrate excess of acid with 0.02 *N* NaOH, using methyl red as indicator. 1 ml of 0.1 *N* H<sub>2</sub>SO<sub>4</sub> = 0.0240 g of ether-soluble alkaloids of ipecac.

**39.122***Hand Extraction Method—Tentative*

(More rapid than automatic extraction method and yields results almost as high.)

Pipet 20 ml of prepared filtrate, 39.120, into separator. Add 2 ml of 8% NH<sub>4</sub>OH soln and extract the soln with equal volumes of peroxide-free ether until extraction is complete (at least 8 times), using Mayer reagent, 39.76, as a test. Wash combined ether extracts in second separator with ca 10 ml of H<sub>2</sub>O, and then wash this wash H<sub>2</sub>O with a little peroxide-free ether, adding ether washings to main soln. Transfer ether soln to Erlenmeyer flask (200 ml flask is convenient size), and evaporate ether on steam bath with aid of blast of air. Add 2–3 ml of absolute alcohol and repeat evaporation to remove all traces of NH<sub>3</sub>. Warm alkaloidal residue with 2–3 ml of neutral alcohol to insure complete soln, and titrate as directed in 39.121.

**IPOMEA (63)—TENTATIVE****39.123****DETERMINATION OF RESIN**

Place 10 g of the drug in a No. 60 powder in Erlenmeyer flask of ca 250 ml capacity and add 50 ml of alcohol. Fit the flask with stopper thru which is inserted glass tube ca 1 m long to act as condenser, and heat on gently simmering steam bath for 30 min., shaking occasionally. Transfer contents of flask to small percolator and percolate slowly with warm alcohol until ca 95 ml of tincture has been obtained. To ascertain whether extraction is complete, collect a further 10 ml of percolate and pour a few drops into cold H<sub>2</sub>O; if more than a faint cloudiness appears, continue the percolation with warm alcohol until test for resin fails. Concentrate the additional percolate by evaporation and add to the flask before making to volume. Cool percolate to room temp. and make to 100 ml with alcohol. Mix well.

Evaporate 25 ml of the prepared tincture (representing 2.5 g of drug) on water bath in beaker or flask of suitable size and dry the residue until it is free from alcohol. Add 15 ml of H<sub>2</sub>O, bring mixture to boiling, allow to cool ca 3 min., and stir well with flat-headed glass rod for 2 min. to insure thoro washing of the resin. Cool mixture by placing container in jar of ice-cold H<sub>2</sub>O and decant wash H<sub>2</sub>O onto 9 cm filter paper. Repeat washing of resin with another 15 ml portion of H<sub>2</sub>O, boiling and cooling the mixture, kneading resin as before, and decanting washings onto filter, as described previously. Repeat washing and kneading process with hot H<sub>2</sub>O a third time. Dissolve residue in container in 10 ml of warm alcohol and pour soln onto the filter, collecting filtrate in weighed beaker or flask. Use sufficient hot alcohol in small portions to completely transfer soln of resin to filter and insure thoro washing of filter. Evaporate combined filtrate and washings to apparent dryness, add 1 ml of absolute alcohol, and evaporate the solvent, taking care to rotate container in inclined position as last portions of solvent are dissipated. Dry residue at 80° to constant weight.

**39.124****JALAP (63)—TENTATIVE**

Proceed as directed under 39.123.

**PODOPHYLLUM (64)—TENTATIVE****39.125****DETERMINATION OF RESIN**

Place 10 g of sample, No. 60 powder, in Erlenmeyer flask of ca 250 ml capacity and add 35 ml of alcohol. Fit flask with stopper thru which is inserted a glass tube

ca 1 m long to act as condenser, and heat mixture on gently simmering steam bath 30 min., shaking occasionally. Transfer contents of flask to small percolator and percolate slowly with hot alcohol until ca 95 ml of percolate has been obtained. Collect ca 10 ml more of the percolate in separate container. Cool first percolate to room temp. and make volume to 100 ml with a portion of second percolate.

Place 50 ml of the alcoholic soln in tared beaker and add 2 ml of  $H_2O$ . Evaporate until percolate weighs 3 g. If weight should fall below 3 g, add alcohol dropwise to make up to 3 g. Pour residue slowly, with constant stirring, into second beaker containing 10 ml of  $H_2O$  previously mixed with 1 ml of normal HCl and cooled to temp. below  $10^\circ$ . (Pellets of ice placed in the beaker and renewed from time to time serve well.) Add 5 ml of  $H_2O$  and a few drops of 10% HCl to the tared beaker, stir well, and rub sides of container with glass rod. Add mixture to the second beaker and allow to stand overnight in refrigerator. Decant supernatant liquid into tared Gooch crucible and transfer precipitate to crucible by means of small portions of cold  $H_2O$  slightly acidulated with HCl. (If preferred, collect precipitate on filter paper and, after washing, dissolve in hot alcohol, collecting soln in tared beaker.) Dry contents of crucible at  $80^\circ$  and weigh. If particles of resin adhere to either beaker, dissolve them in alcohol, evaporate solvent in tared beaker, and dry residue at  $80^\circ$ . Cool, weigh, and add total net weight to weight of contents of crucible.

#### BELLADONNA AND STRAMONIUM OINTMENTS (85)—TENTATIVE

##### 39.126

##### *Method I*

Weigh ca 25 g of the well-mixed ointment into 250 ml separator fitted with pledget of cotton packed loosely in stem; add 100 ml of ether- $CHCl_3$  mixture (4+1) and shake vigorously until all fats are dissolved. Extract the alkaloids by shaking out with 5 successive 20 ml portions of dilute  $H_2SO_4$  (2% is satisfactory), allow to settle, and draw off clear acid soln into small separator containing 10 ml of ether. Wash each acid extraction successively thru this same 10 ml of ether and draw off the acid solns into another 250 ml separator. Make combined acidified solns alkaline with  $NH_4OH$  and extract alkaloids completely by shaking out with 5 successive 25 ml portions of  $CHCl_3$ . Each time allow to settle, then filter  $CHCl_3$  thru cotton wetted with  $CHCl_3$  into 250 ml beaker, finally washing stem of separator and filter with a little  $CHCl_3$ . Evaporate solvent carefully on water bath with moderate heat to volume of ca 10 ml; add measured excess (ca 10 ml) of 0.02 N  $H_2SO_4$ , stir mixture, and continue evaporation until all  $CHCl_3$  has been expelled. Add 20 ml of recently boiled, cooled  $H_2O$  and one drop of methyl red indicator and titrate excess acid with 0.02 N NaOH. 1 ml of 0.02 N  $H_2SO_4$  = 0.00579 g of the alkaloids of belladonna or stramonium leaves.

NOTE: It is recommended that the ointment be transferred by means of a soft metal ointment tube or empty tooth paste tube, and weighed by difference. The assay can be hastened by centrifuging where instructions are given to let the mixture stand until it settles.

##### 39.127

##### *Method II*

Weigh ca 25 g of the ointment into tall-form beaker. Add ca 5 g of paraffin, 25 ml of 2%  $H_2SO_4$ , and 10 ml of ether. Warm gently on steam bath until fluid, stirring mixture thoroly. Continue this procedure until most of ether has been evaporated. Place beaker in ice bath and allow to stand until cold. Make several holes in paraffin layer with stirring rod and filter acid soln thru pledget of cotton into small separator. Wash the cake once with small quantity of  $H_2O$ , filtering washings thru cotton into separator. Wash the acid with 10 ml of ether and draw off into 250 ml separator.

Repeat treatment with 4 successive portions of acid and ether, filtering each portion thru the cotton into the small separator and washing each extraction with the same 10 ml portion of ether. Combine the acidified extractions, make alkaline with 10%  $\text{NH}_4\text{OH}$ , and extract and titrate the alkaloids as directed in 39.126.

## 39.128

## MENTHOL (66)—OFFICIAL

Weigh 5 g of menthol in 100 ml acetylation flask, and add 10 ml of acetic anhydride and 1 g of powdered anhydrous Na acetate. Boil mixture gently 1 hour, cool, and disconnect flask from condenser, transferring mixture to small separator. Rinse acetylation flask with 3 successive 5 ml portions of warm  $\text{H}_2\text{O}$  and add rinsings to separator. When liquids have completely separated, remove aqueous layer, and wash remaining oil with successive 5 ml portions of  $\text{Na}_2\text{CO}_3$  soln (12.5 g in 200 ml of  $\text{H}_2\text{O}$ ), until mixture is alkaline to 2 drops of phenolphthalein soln. Dry resulting oil with fused  $\text{CaCl}_2$  and filter. Transfer 4–5 ml of the dry acetylated oil to tared 100 ml Erlenmeyer flask, note exact weight, add 50 ml of 0.5 *N* alcoholic KOH, connect flask to reflux condenser, and boil mixture on water bath 1 hour. Allow mixture to cool, disconnect flask from condenser, and titrate excess of alkali with 0.5 *N*  $\text{H}_2\text{SO}_4$ , using 10 drops of phenolphthalein soln as indicator. Calculate percentage of menthol by following formula:

$$\text{Percentage of total menthol} = \frac{A \times 7.813}{B - (A \times 0.021)}, \text{ in which}$$

*A* is result obtained by subtracting number of ml of 0.5 *N*  $\text{H}_2\text{SO}_4$  required in titration from number of ml of 0.5 *N* alcoholic KOH originally taken, and *B* is weight of acetylated oil taken.

## THYMOL (67)—OFFICIAL

## 39.129

## PREPARATION OF SOLUTION

Weigh 2 g of pulverized thymol, transfer to 500 ml volumetric flask, and add 25 ml of 25% NaOH soln. Agitate until the thymol is dissolved and dilute to mark at 20° with  $\text{H}_2\text{O}$ .

## DETERMINATION

## 39.130

## Method I

Transfer 25 ml aliquot of the thymol soln to 250 ml glass-stoppered Erlenmeyer flask, add 20 ml of hot HCl (1+1), and immediately run in 1–3 ml less than the theoretical amount of 0.1 *N* KBr-KBrO<sub>3</sub>, 39.28(c). Warm to 70–80°, add 2 drops of methyl orange soln (0.1 g in 100 ml of  $\text{H}_2\text{O}$ ), and titrate slowly with the KBr-KBrO<sub>3</sub> soln, swirling vigorously after each addition. When the red color of the methyl orange is bleached, add 2 drops of the titrating soln, stopper, shake vigorously 10 seconds, add a drop of the methyl orange soln, and again shake vigorously 10 seconds. Continue the addition of 2 drops of the KBr-KBrO<sub>3</sub> soln, shaking until the red color disappears. Add 1 drop of the methyl orange soln, shake vigorously, and if the red color does not disappear, repeat the alternate addition of 2 drops of KBr-KBrO<sub>3</sub> soln and 1 drop of methyl orange soln, shaking after each addition as directed previously, until the red color disappears. Calculate number of ml of KBr-KBrO<sub>3</sub> soln used to percentage of thymol. 1 ml of 0.1 *N* KBr-KBrO<sub>3</sub> = 0.003753 g of thymol. Reserve mixture in titrating flask for 39.131.



## 39.131

*Method II*

To the cooled mixture resulting from the titration, **39.130**, add 3–5 ml additional KBr-KBrO<sub>3</sub> soln. Stopper, shake, add 1 g of solid KI, wash sides of flask and stopper with H<sub>2</sub>O, and titrate the I liberated by the excess KBr-KBrO<sub>3</sub> soln with 0.1 *N* Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, using starch soln, **6.3(e)**, as indicator. Calculate amount of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> used in terms of KBr-KBrO<sub>3</sub> soln, deduct from total amount of KBr-KBrO<sub>3</sub> soln added, and calculate to percentage of thymol.

To determine approximate number of ml of KBr-KBrO<sub>3</sub> soln required for Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, heat a 25 ml aliquot of the sample and 20 ml of HCl (1+1) to ca 80° and titrate slowly with the KBr-KBrO<sub>3</sub> soln, swirling vigorously while titrating until a yellow color, permanent for 1 min., appears.

## 39.132

**THYMOL IN ANTISEPTICS (68)—OFFICIAL**

If alcoholic content is not known, make preliminary determination of alcohol.

Transfer 50 ml of sample (or aliquot containing 0.05–0.10 g of thymol) to a Pt or porcelain evaporating dish. Add 6–7 ml of 50% NaOH soln, mix well, and carefully dealcoholize by placing dish on steam bath before electric fan. Evaporate a volume slightly more than quantity of alcohol present. (If over 30% of alcohol is present, dilute with H<sub>2</sub>O to an alcoholic content of 25%. In no case should the evaporation be carried beyond 70% of original volume.) Transfer soln to a 125 ml separator, washing out evaporating dish with sufficient H<sub>2</sub>O to bring the volume to ca 75 ml.

Extract the alkaline soln twice with petroleum benzine, using 20 ml each time. Wash the extracts once with 5–10 ml of 5% NaOH soln and add washings to aqueous layer. Extract aqueous alkaline soln containing the thymol, together with Na salts of boric, benzoic, and salicylic acids, with ether, making 5 extractions (20, 15, 15, 10, 10 ml). Use 8 to 10 extractions if the preparation contains glycerol. Combine the ether extracts, transfer to 250 ml glass-stoppered Erlenmeyer flask, add 5 ml of recently prepared alcoholic KOH soln, **31.24**, and evaporate most of the ether, using steam bath and electric fan. Do not evaporate entirely to dryness but leave 6–8 ml residue. To this residue add 75 ml of hot H<sub>2</sub>O (80–90°) and 10 ml of HCl.

Immediately run in 1–3 ml less than the theoretical quantity of 0.1 *N* KBr-KBrO<sub>3</sub>, **39.28(c)**, swirling contents of flask constantly. Add 2 drops of methyl orange soln and titrate slowly with the KBr-KBrO<sub>3</sub> soln, shaking vigorously after each addition. When the red color of the methyl orange is bleached, add 2 drops of the titrating soln, stopper, shake vigorously 10 seconds, add one drop of methyl orange soln, and again shake vigorously 10 seconds. Continue the addition of the KBr-KBrO<sub>3</sub> soln, 2 drops at a time, shaking after each addition until red color disappears. Add 1 drop of methyl orange soln, shake vigorously, and if the red color does not disappear, repeat alternate addition of 2 drops of the KBr-KBrO<sub>3</sub> soln and 1 drop of methyl orange soln, shaking after each addition, as directed above, until red color disappears. 1 ml of 0.1 *N* KBr-KBrO<sub>3</sub> = 0.003753 g of thymol.

Test for complete extraction by shaking out the aqueous layer twice with 15–20 ml of ether and titrating the thymol, if any, in the ether extracts. Add this titration to that obtained for the main ether extract. If the theoretical amount of thymol present is not known, add 2 drops of methyl orange soln, and titrate slowly, swirling constantly during the addition of KBr-KBrO<sub>3</sub> soln until the red color is bleached. Continue according to method outlined, beginning “add 2 drops of the titrating soln, stopper, shake vigorously etc.”

**CAUTION:** To avoid loss of thymol by volatilization, both the evaporation of alcohol and later evaporation of ether must be done carefully.

**39.133 VOLATILE ACIDITY OF TRAGACANTH (69)—OFFICIAL, FIRST ACTION**

The quantity of volatile (acetic) acidity developed in the acid hydrolysis of gum tragacanth (*Astragalus gummifer* Lab.) affords a valuable index of purity of this commodity when compared with results obtained by similar treatment of so-called "Indian gum" (*Cochlospermum gossypium* D. C. and *Sterculia urens* Roxb.).

Treat 1 g of whole or powdered sample in 700 ml round-bottomed, long-necked flask in the cold with 100 ml of  $H_2O$  and 5 ml of  $H_3PO_4$  for several hours, or until the gum is completely swollen. Boil gently for 2 hours under reflux condenser. A very small quantity of cellulose substance will remain undissolved. Tragacanth yields a practically colorless soln. Indian gum gives a pink or rose soln. This reaction may be used as a preliminary test for detection of Indian gum.

Distil the hydrolyzed product with steam, using a scrubber (Fig. 61) to connect distillation flask with condenser. Continue distillation until distillate amounts to 600 ml, and the acid residue to ca 20 ml. To avoid scorching of residue do not permit concentration of contents of distilling flask to less than 20 ml. Titrate distillate with 0.1 N NaOH, using 10 drops of phenolphthalein indicator, 2.10(d). Correct result by blank determination and express as "volatile acidity" the number of ml of 0.1 N NaOH required to neutralize the volatile (acetic) acid obtained.

**CHLOROBUTANOL (70)—TENTATIVE****39.134****REAGENTS**

(a) *Alcoholic potassium hydroxide soln.*—See 39.136(a).

(b) *Silver nitrate soln.*—Dissolve 10 g of  $AgNO_3$  in sufficient  $H_2O$  to make 500 ml.

**39.135****DETERMINATION**

(a) *Chlorobutanol crystals.*—Transfer to pressure bottle a sample equivalent to ca 0.3 g of chlorobutanol and carefully add 25 ml of the alcoholic KOH soln. Stopper bottle, and mix contents by gentle swirling, taking care to prevent soln from coming in contact with rubber washer, then allow to stand 30 min. or overnight. Place bottle in wire basket, and set basket in water bath at room temp. Invert a tin can over bottle and cover with towel to prevent injury in case bottle should burst. Heat bath to boiling and maintain this temp. 15 min. Cool gradually.

Add 25 ml of  $H_2O$ , swirling gently; and transfer contents of pressure bottle to 400 ml beaker. Wash bottle with  $H_2O$ , draining washings into beaker. Add 15 ml of  $HNO_3$  and an excess of the  $AgNO_3$  soln, stir well, and allow mixture to stand in dark place 15 min. Collect precipitate in Gooch crucible that has been dried at  $105^\circ$  and weighed. Wash precipitate thoroly with  $H_2O$ , then with 5 ml of alcohol followed by a 5 ml portion of ether. Dry to constant weight at  $105^\circ$ . If reagents contain Cl, apply correction determined by blank test.  $1\text{ g of }AgCl = 0.4127\text{ g of }C_4H_7OCl_2$ .

(b) *Ampul solns.*—Pipet into a distilling flask a sample equivalent to ca 0.1 g of chlorobutanol. Add sufficient  $H_2O$  to bring volume to 50 ml and distil ca 25 ml thru a straight-bore condenser. Collect distillate in pressure bottle of ca 100 ml capacity containing 25 ml of the alcoholic KOH and surrounded by ice bath. Have delivery tube extend into the alcoholic soln. (Use a straight-bore condenser to assure complete soln of the crystals of chlorobutanol in the condenser.) Allow to cool, disconnect still head, and wash condenser carefully with 25 ml of alcohol, allowing alcohol to drain into pressure bottle. Repeat washing, using ca 20 ml of  $H_2O$ . Also wash the receiving tube with  $H_2O$ . Stopper pressure bottle and mix contents by gentle swirling, taking care to prevent soln from coming in contact with rubber washer. Allow to stand 30 min. or overnight. Complete determination of Cl as directed in (a).

## CHLOROFORM AND CARBON TETRACHLORIDE (71)—OFFICIAL

## 39.136

## REAGENTS

(a) *Alcoholic potassium hydroxide soln.*—Dissolve 35 g of KOH (free from Cl) in sufficient methyl alcohol to make 100 ml. Allow to stand several days and decant the clear liquid.

(b) *Ammonium thiocyanate soln.*—0.05 *N*. Adjust by titrating against 0.1 *N* AgNO<sub>3</sub>.

(c) *Ferric ammonium sulfate indicator.*—Dissolve 8 g of FeNH<sub>4</sub>(SO<sub>4</sub>)<sub>2</sub> · 12H<sub>2</sub>O in sufficient H<sub>2</sub>O to make 100 ml.

## 39.137

## WEIGHING OF SAMPLE

(1) *Chloroform or Carbon Tetrachloride.*—Carefully transfer 30 ml of alcoholic KOH soln to an air-dried, 60–70 ml pressure bottle, and stopper. Do not moisten neck of bottle with the reagent. Weigh stoppered bottle with contents (conveniently done by suspending bottle on balance by means of the clamp that holds stopper).

Immediately after opening bottle, add ca 1 ml of sample from 1 ml pipet, holding pipet just above top level of reagent in pressure bottle. As level of reagent rises with draining of sample into bottle, raise pipet correspondingly so as to avoid contact with reagent. Avoid having bottle open longer than necessary, 20 seconds being convenient time. Stopper bottle so as to assure a tight fit and weigh. Determine weight by difference. Proceed as directed under 39.138.

(2) *Carbon Tetrachloride in Capsules.*—Ascertain gross weight of representative number of capsules. Open capsules and transfer contents to suitable flask. Weigh dried empty capsules and determine average net contents. Proceed as directed under (1), using the composite sample.

(3) *Chloroform or Carbon Tetrachloride in Mixtures.*—Proceed as directed under (1), using not more than 10 ml of the mixture containing 0.08–1.6 g of CHCl<sub>3</sub> or CCl<sub>4</sub>. Note temp. of mixtures. Ascertain volume-equivalent of weighed sample. Weigh definite volume of mixture at same temp., using a 50 or 100 ml volumetric flask, and calculate.

NOTE: If desired, sample may be measured directly with a pipet instead of being weighed, or a measured volume may be diluted with methyl alcohol to some definite volume, thoroly mixed, and a suitable aliquot of this dilution used.

## 39.138

## DETERMINATION

If sample is a mixture, mix contents of bottle by gentle swirling and allow bottle to stand ca one hour (30 min. is sufficient for CHCl<sub>3</sub>, pure or nearly so). Place bottle in a wire basket and set basket in water bath at room temp. Invert a tin can over bottle and cover with towel to prevent injury to analyst in case bottle should burst. Heat bath to boiling and maintain at this temp. 1 hour (15 min. is sufficient for CHCl<sub>3</sub>, pure or nearly so). Cool contents of pressure bottle gradually, transfer to 200 ml volumetric flask, and wash out bottle thoroly with H<sub>2</sub>O, draining washings into flask. Bring to room temp., fill to mark with H<sub>2</sub>O, and mix.

Transfer suitable aliquot to 100 ml volumetric flask and acidify with HNO<sub>3</sub>, adding ca 2 ml in excess. Add 25 or 50 ml of 0.1 *N* AgNO<sub>3</sub> (an excess), shake thoroly, fill to mark with H<sub>2</sub>O, and mix. Filter mixture thru dry filter into dry flask, rejecting first 20 ml of filtrate. To 50 ml aliquot of filtrate, add 3 ml of the Fe(NH<sub>4</sub>)(SO<sub>4</sub>)<sub>2</sub> indicator and titrate the excess AgNO<sub>3</sub>, using 0.05 *N* NH<sub>4</sub> or K thiocyanate.

If original sample contains chloride, determine quantity and make correction. If original sample contains sugar or other organic material and (after saponification of the CHCl<sub>3</sub> or CCl<sub>4</sub> and dilution of mixture with H<sub>2</sub>O) is highly colored,

thus interfering with titration, transfer contents of pressure bottle to Ni crucible with aid of  $\text{H}_2\text{O}$ . Evaporate to dryness and char residue. Allow to cool, treat with  $\text{H}_2\text{O}$ , filter into suitable volumetric flask, and wash residue and filter with  $\text{H}_2\text{O}$  until free from Cl. Fill to mark with  $\text{H}_2\text{O}$ , mix, and determine Cl as directed previously.

Make a blank test, using in pressure bottle the same quantities of solvents and reagents as with sample, and apply necessary correction. 1 ml of 0.1  $N$   $\text{AgNO}_3$  = 0.00398 g of  $\text{CHCl}_3$  or 0.00385 g of  $\text{CCl}_4$ .

#### TETRACHLORETHYLENE IN MIXTURES (72)—OFFICIAL

##### 39.139

##### REAGENTS

(a) *Metallic sodium*.—Place 10 ml of xylene and 2 g of metallic Na in a small Erlenmeyer flask fitted with glass stopper, adding more xylene if necessary to cover metal. Heat on hot plate until the Na is melted. Shake to remove excess vapor, stopper, wrap in towel, and shake vigorously until the Na is finely divided. Cool, remove xylene, and replace with 5 ml of fresh xylene.

(b) *Ferric ammonium sulfate indicator*.—See 39.136 (c).

##### 39.140

##### DETERMINATION

Weigh carefully a 125 ml cork-stoppered Erlenmeyer flask. Remove from balance pan, open, and from a split ml pipet add sufficient sample to give equivalent of ca 0.16 g of tetrachlorethylene. Stopper securely and weigh again. To contents of flask add 10 ml of xylene and 2 g of the Na reagent. Connect flask to reflux condenser, using cork stopper protected by tin foil, and heat on hot plate to boiling. Add ca 1 ml of amyl alcohol thru condenser. Reflux gently 2 hours and add at intervals 1 ml portions of amyl alcohol until a total of 5 ml is added. Disconnect flask. When cool, destroy excess of Na by cautious addition of 20 ml of  $\text{H}_2\text{O}$ . After all action has subsided, acidify with  $\text{HNO}_3$  and transfer mixture to separator. Wash xylene layer with three 10 ml portions of  $\text{H}_2\text{O}$  and filter the acid aqueous solns into 200 ml volumetric flask. Add 50 ml of 0.1  $N$   $\text{AgNO}_3$  to flask and make up to 200 ml. Shake thoroly and pour thru dry filter, discarding first 20 ml of filtrate. To 100 ml aliquot add 3 ml of the indicator. Titrate excess  $\text{AgNO}_3$ , using 0.05  $N$   $\text{NH}_4\text{CNS}$ . Make a blank test for chloride. 1 ml of 0.1  $N$   $\text{AgNO}_3$  = 0.00415 g of  $\text{C}_2\text{Cl}_4$ . The Cl may also be determined gravimetrically. 1 g of  $\text{AgCl}$  = 0.2892 g of  $\text{C}_2\text{Cl}_4$ .

#### ETHER (73)—OFFICIAL

(Not applicable in presence of essential oils)

##### 39.141

##### REAGENTS

(a) *Sulfuric acid*.—(1+1). Carefully add  $\text{H}_2\text{SO}_4$  to an equal volume of  $\text{H}_2\text{O}$  and cool to room temp.

(b) *Potassium dichromate soln*.—1  $N$ . Dissolve 49.035 g of pure  $\text{K}_2\text{Cr}_2\text{O}_7$  (or corresponding quantity of known purity) in sufficient  $\text{H}_2\text{O}$  to make 1 liter.

(c) *Sulfuric acid-potassium dichromate soln*.—0.5  $N$ . Carefully add 500 ml of  $\text{H}_2\text{SO}_4$  to 500 ml of 1  $N$   $\text{K}_2\text{Cr}_2\text{O}_7$  (accurately measured in volumetric flask), and cool to room temp. Use two 1 liter flasks for mixing and cooling. Transfer to 1 liter volumetric flask, using  $\text{H}_2\text{SO}_4$  for washing, and fill to mark with  $\text{H}_2\text{SO}_4$ . Mix thoroly. Standardize against 0.05  $N$   $\text{Na}_2\text{S}_2\text{O}_3$  soln as follows:

Pipet exactly 25 ml of Reagent (c) into a 250 ml glass-stoppered volumetric flask and dilute to mark with  $\text{H}_2\text{O}$  after cooling to room temp. Mix thoroly. Pipet 50 ml

aliquot into 500 ml glass-stoppered flask; add 100 ml of  $\text{H}_2\text{O}$ , 10 ml of  $\text{H}_2\text{SO}_4$ , and 10 ml of 25% KI soln, freshly prepared. Stopper flask and allow to stand 3–5 min. Add 150–200 ml of  $\text{H}_2\text{O}$  and titrate with 0.05 N  $\text{Na}_2\text{S}_2\text{O}_8$ , using starch soln, 6.3(e), freshly prepared, as indicator.

## 39.142

## APPARATUS

Set up apparatus as illustrated, Fig. 66. Beginning at air intake end of aspiration train, use a 400 ml bottle as wash bottle (A), six 50 ml graduated cylinders, having an inside diameter of 1.5 cm and a height of 32–35 cm (B–C–D–E–F–G), a 500 ml bottle as safety reservoir (H), and a 400 ml bottle as wash bottle (I), which is supplied with a soda-lime tube. Supply each container with a closely fitting rubber stopper and vapor-carrying tubes. Have intake tube extend almost to bottom, and have outlet tube 1 cm below the rubber stopper. Use heavy-walled glass tubing having outside diameter of 5 mm. Draw outlets of all vapor-carrying tubes down to small openings. Use heavy walled rubber tubing for connections and between cylinders expose only 0.5–1 cm to the vapors.

## 39.143

## PREPARATION OF SAMPLE

Carefully weigh 100 ml glass-stoppered volumetric flask containing 65–70 ml of  $\text{H}_2\text{O}$ . Pipet 5 ml of ether, holding pipet just above the  $\text{H}_2\text{O}$  in the flask, and as the level of the  $\text{H}_2\text{O}$  is raised by the draining of the ether into the flask raise pipet correspondingly to avoid contact with the  $\text{H}_2\text{O}$ . Immediately stopper flask and weigh. Difference in weight is weight of ether. Carefully and gently swirl liquid in flask until ether is dissolved and then fill to mark with  $\text{H}_2\text{O}$ . Stopper flask and thoroly mix. If unknown ether sample is an alcoholic or hydroalcoholic soln, prepare a soln by dilution with  $\text{H}_2\text{O}$  to meet requirements under 39.145.

## 39.144

## PRELIMINARY CHARGING OF APPARATUS

Transfer ca 100 ml of the  $\text{H}_2\text{SO}_4$ - $\text{K}_2\text{Cr}_2\text{O}_7$  soln to wash bottle A, and 35 ml of the  $\text{H}_2\text{SO}_4$  soln to each cylinder, C and D. (Use a funnel with a long stem to avoid wetting upper portion of container.) Pipet 40, 25, and 25 ml of the  $\text{H}_2\text{SO}_4$ - $\text{K}_2\text{Cr}_2\text{O}_7$  soln into cylinders E, F, and G, respectively, avoiding unnecessary wetting of outside of stem of pipet and touching inside of cylinder with the wetted stem of the pipet while draining. Bottle H remains empty. Transfer ca 50 ml of  $\text{H}_2\text{SO}_4$  to bottle I and fill tube J with an appropriate quantity of soda-lime, layered on bottom and top with cotton. Stopper tightly all containers except cylinder B. Leave all rubber tubing connections between cylinders and glass stopcocks K and L open.

## 39.145

## DETERMINATION

If sample is known not to contain alcohol or other substances that will be oxidized by the  $\text{H}_2\text{SO}_4$ - $\text{K}_2\text{Cr}_2\text{O}_7$  soln, pipet an aliquot as directed in 39.144 into 250 ml ground-glass-stoppered flask containing 50 ml of the same reagent. Stopper flask, swirl gently, and allow to stand 1 hour. Titrate excess acid  $\text{K}_2\text{Cr}_2\text{O}_7$  and calculate as directed below.

Pipet an aliquot of the sample containing 0.035–0.2 g of ether in aqueous soln or hydroalcoholic soln, containing not more than 5 g of alcohol, into cylinder B containing sufficient  $\text{H}_2\text{O}$  to make a total volume of 25 ml. Hold pipet just above top level of liquid in cylinder, and as the liquid is raised by the draining of the sample, raise pipet correspondingly so as to avoid contact with the liquid.

Stopper tightly and immediately connect with cylinder C and wash bottle A. Connect the suction pump at M, and with stopcock L about half open start the pump. With bottle H and cylinder G connected, gradually close stopcock K until a slow current of bubbles passes thru the reagent in cylinder F, and connect cylinder E. Repeat until cylinder B, which contains the sample, is connected. Make certain all connections are air-tight. (Usually stopcock L requires no further adjustment.)

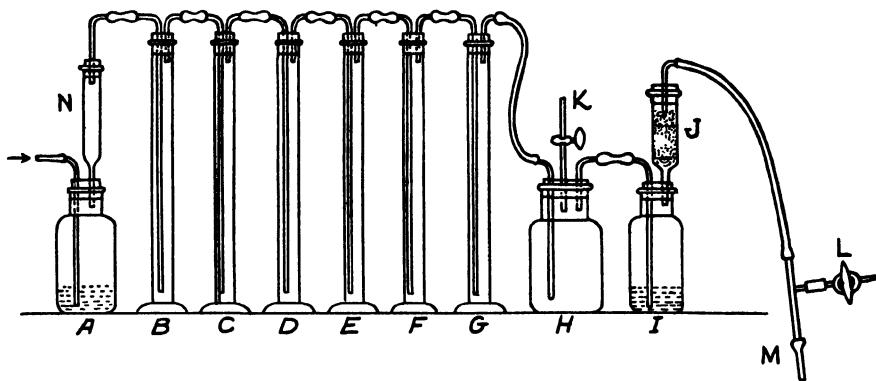


FIG. 66.—APPARATUS FOR DETERMINATION OF ETHER

Carefully adjust stopcock K until a rapid and steady current of bubbles (ca 150 per min.) flows thru the aspiration train. (Usually this is attained with cock K slightly open, depending upon size of opening thru cock L.) Use care not to have any of the reagent touch the rubber stopper by spray or otherwise. As the bubbles rise in cylinders B and C they increase in size, couple up, and near the surface each bubble occupies the entire cross-section of the cylinder and has a vertical height of 1–1.5 cm.

Aspirate for 5 hours. If not certain that all the ether has been carried over into the 0.5 *N* acid  $K_2Cr_2O_7$  soln, discontinue the aspiration as directed in the following paragraph. Transfer contents of cylinder E to a ground-glass-stoppered 500 ml volumetric flask. Pipet 25 ml of acid  $K_2Cr_2O_7$  soln into cylinder E. Aspirate as before.

Gradually open cock K until rate of flow of bubbles is appreciably slower and disconnect rubber tubing between cylinders B and C. Gradually open cock K as before and disconnect the tubing between C and D. Repeat until all cylinders are disconnected.

Transfer the acid  $K_2Cr_2O_7$  soln (contents of cylinders E, F and G) to a ground-glass-stoppered 500 ml volumetric flask. Wash cylinders and glass tubes with  $H_2O$  and drain washings into flask. Add 200–300 ml of  $H_2O$  and cool. Add more  $H_2O$  and again cool to room temp. Make up to volume and mix thoroly. Pipet a 25 ml aliquot into a 500 ml ground-glass-stoppered flask and continue as directed under 39.141(c), beginning “add 100 ml of  $H_2O$ .” Calculate 0.5 *N*  $H_2SO_4$ - $K_2Cr_2O_7$  soln consumed by the sample. 1 ml of 0.5 *N* acid  $K_2Cr_2O_7$  = 0.00463 g of ether.

#### CINCHOPHEN

##### *I. In Presence of Salicylates (74)—Official, First Action*

#### 39.146

##### REAGENTS

(a) *Sodium carbonate soln.*—Dissolve 12.5 g of  $Na_2CO_3 \cdot H_2O$  in sufficient  $H_2O$  to make 100 ml.

- (b) *Iodine soln.*—0.1 N. See 39.212.  
 (c) *Sodium thiosulfate soln.*—0.02 N. See 39.3(b).  
 (d) *Starch indicator.*—See 6.3(e).

## 39.147

## DETERMINATION

If the product is a solid, weigh into 50 ml beaker sufficient finely powdered sample to contain ca 0.15 g of cinchophen. Treat with 5, 3, and 3 ml portions of the  $\text{Na}_2\text{CO}_3$  soln, and filter thru small (5 cm) paper into 50 ml beaker, finally washing first beaker and paper with a little  $\text{H}_2\text{O}$ . Evaporate filtrate and washings to complete dryness on steam bath with aid of air blast. If product is in form of a clear soln, transfer measured portion to beaker, and evaporate to dryness. In either case, dissolve hot residue in 5 ml of acetic acid and transfer to 100 ml volumetric flask, using not more than 10 ml of the acid to complete the transfer. Heat to ca  $90^\circ$  on steam bath. Add 25 ml of the I soln slowly from a pipet with constant agitation of flask, and immediately stopper flask. Allow to cool, dilute to 100 ml with  $\text{H}_2\text{O}$ , stopper, and let stand for 30 min. with occasional thoro agitation. Filter thru small, rapid filter, rejecting first 15 ml of filtrate, and immediately titrate a 50 ml aliquot with the standard thiosulfate soln, adding the starch indicator as end point is approached. 1 ml of 0.1 N I = 0.0166 g of cinchophen,  $\text{C}_{10}\text{H}_{11}\text{O}_2\text{N}$ .

*II. In Presence of Sodium Bicarbonate (75)—Official, First Action*

## 39.148

## REAGENTS

- (a) *Solvent.*—Mix 50 ml of alcohol and 50 ml of ether with 100 ml of  $\text{CHCl}_3$ .  
 (b) *Neutral alcohol.*—Neutralize to phenolphthalein with 0.1 N NaOH.

## 39.149

## DETERMINATION

Weigh sufficient powdered sample to yield 0.3–0.4 g of cinchophen, transfer to separator, and add 10 ml of 4% NaOH soln to dissolve the cinchophen. Neutralize with 10% HCl and add 2 ml in excess. Extract with five 25 ml portions of the solvent, collecting extracts in second separator. Wash with 25 ml of  $\text{H}_2\text{O}$  and filter extracts into beaker. Extract wash  $\text{H}_2\text{O}$  with 15 ml of solvent and filter into same beaker. Test for complete extraction. Evaporate solvent to dryness on steam bath. Dissolve residue in 60 ml of neutral alcohol. Titrate the soln with 0.1 N NaOH to a permanent pink color, using phenolphthalein as indicator. 1 ml of 0.1 N NaOH = 0.02491 g of cinchophen.

**DINITROPHENOL (OR ITS SODIUM COMPOUND) (76)—OFFICIAL**

## 39.150

## REAGENTS

- (a) *Sodium hydroxide soln.*—Dissolve 2 g of NaOH in 100 ml of  $\text{H}_2\text{O}$ .  
 (b) *Potassium iodide soln.*—Dissolve 20 g of KI in 100 ml of  $\text{H}_2\text{O}$ .  
 (c) *Bromide-bromate soln.*—0.1 N. Dissolve 2.7835 g of  $\text{KBrO}_3$  and 12 g of KBr in  $\text{H}_2\text{O}$  and dilute to 1 liter. If necessary, standardize against 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$  as in 39.28(c).  
 (d) *Sodium thiosulfate soln.*—0.1 N. See 39.3(b).

## 39.151

## DETERMINATION

(a) *Interfering substances absent.*—Weigh 0.18–0.20 g of 2,4 dinitrophenol (or sufficient of the preparation to contain that quantity) into 100 ml beaker, and dissolve in 25 ml of  $\text{H}_2\text{O}$ , using sufficient 2% NaOH soln to insure soln. Transfer soln to 500 ml glass-stoppered flask, using  $\text{H}_2\text{O}$  for washing (do not use heat). Dilute with

H<sub>2</sub>O to ca 100 ml and add 25 ml of the KBr-KBrO<sub>3</sub> soln and 10 ml of HCl. Immediately stopper flask and swirl vigorously 1-3 min. Remove stopper quickly and add 5 ml of KI soln, taking care to avoid loss of Br; immediately stopper flask and shake thoroly ca 1 min. Remove stopper and rinse down neck of flask with H<sub>2</sub>O. Titrate with the Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln, using starch indicator, 6.3(e), near end point.

1 ml. of 0.1 *N* KBr-KBrO<sub>3</sub> = 0.0092 g of 2,4 dinitrophenol.

1 ml of 0.1 *N* KBr-KBrO<sub>3</sub> = 0.0103 g of Na dinitrophenate.

1 ml of 0.1 *N* KBr-KBrO<sub>3</sub> = 0.0112 g of Na dinitrophenate monohydrate.

(b) *Interfering substances present.*—Weigh into separator a sample equivalent to ca 0.18 g of 2,4 dinitrophenol or its Na compound. Macerate for a short time with 10 ml of H<sub>2</sub>O and 10 ml of NaOH soln. Acidify with HCl. Extract with 10-20 ml of CHCl<sub>3</sub> and repeat until extraction is complete (usually 5 or 6 extractions are necessary), avoiding vigorous shaking, particularly during first 2 extractions. Test for complete extraction by shaking out the last CHCl<sub>3</sub> extraction with 5 ml of the NaOH soln (a yellow color in latter indicates incomplete extraction; 5 ml containing 0.025 mg is pale yellow).

Combine the CHCl<sub>3</sub> extracts in separator and shake out with 10-15 ml of the NaOH soln. Draw off CHCl<sub>3</sub> layer into third separator and repeat extraction until no more yellow color is extracted. Note total volume of NaOH soln used. Transfer the alkaline solns to 500 ml glass-stoppered flask, washing separator each time with H<sub>2</sub>O. Add exact quantity of HCl necessary, previously determined, to neutralize the NaOH soln used. Proceed as directed under (a).

#### GUAIACOL (77)—TENTATIVE

##### 39.152

##### REAGENT

*Hydriodic acid.*—Sp. gr. 1.7. Boil the HI under reflux condenser with excess of hypophosphorous acid 30 min. When cool, transfer to dark, glass-stoppered bottle. Do not allow acid to stand with stopper removed for more than few minutes.

##### 39.153

##### APPARATUS

*Methoxyl apparatus.*—See Fig. 68, Chap. 41.

##### 39.154

##### DETERMINATION

Introduce an aliquot of the alkaline guaiacol soln (guaiacol dissolved in 1% NaOH) containing 0.03-0.06 g of guaiacol into the boiling flask and evaporate the soln just to dryness on steam bath in current of air. For solid guaiacol compounds weigh 0.06-0.1 g and introduce directly into flask. Complete the determination by the method for methoxyl group, 41.2, beginning with the expression "together with a boiling rod." Boil for 30 min. and use 0.1 *N* Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> for the titration.

1 ml of 0.1 *N* I = 0.00207 g of guaiacol; 0.00228 g of guaiacol carbonate; and 0.00404 g of K guaiacol sulfonate.

#### HEXYLRESORCINOL (78)—TENTATIVE

##### 39.155

##### REAGENTS

(a) *Bromide-bromate soln.*—0.1 *N*. See 39.28(c).

(b) *Sodium thiosulfate soln.*—0.1 *N*. See 39.3(b).

(c) *Purified methanol.*—Add sufficient Br vapor to commercial methanol to give a bright yellow color and heat to boiling on water bath 5 min. Cool, and carefully decolorize by adding 10% NaHSO<sub>3</sub> soln dropwise until methanol is just colorless.



(d) *Potassium iodide soln.*—Dissolve 20 g of KI in H<sub>2</sub>O and dilute to 100 ml.

## 39.156

## STANDARDIZATION OF THIOSULFATE

Add 30 ml of the KBr-KBrO<sub>3</sub> soln to a 150 ml glass-stoppered flask. Add 10 ml of methanol. Wet stopper. Add 5 ml of HCl, stopper flask, immediately place under running tap H<sub>2</sub>O, and swirl until flask cools to room temp. Continue to shake flask 5 min. after HCl is added. Cautiously loosen stopper and add .5 ml of the KI soln. Swirl gently to liberate the I, wash stopper, and titrate with the Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln. Add starch paste when color of soln is pale yellow.

## 39.157

## DETERMINATION

Transfer 0.07–0.09 g of sample to 150 ml glass-stoppered flask. Add 10 ml of methanol and swirl gently to dissolve sample. Add 30 ml of the KBr-KBrO<sub>3</sub> soln. Moisten stopper. Add 5 ml of HCl, stopper flask, and immediately hold under running H<sub>2</sub>O while swirling somewhat vigorously. When cooled to room temp. (ca 1 min.), remove from tap and continue to shake vigorously 5 min. after the HCl is added. Cautiously loosen stopper and add 5 ml of the KI soln. Swirl gently, wash stopper with a little H<sub>2</sub>O, add 1 ml of CHCl<sub>3</sub>, and titrate with Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln while swirling flask gently. Near end point, stopper flask and shake vigorously to get the free halogen out of the CHCl<sub>3</sub>. When color has been reduced to pale yellow, add starch paste and continue titration. End point is reached when starch-I color does not return during 30 seconds' vigorous shaking. 1 ml of 0.1 N KBr-KBrO<sub>3</sub> soln = 0.00488 g of hexylresorcinol.

## MANDELIC ACID (79)—OFFICIAL

## 39.158

*Qualitative Tests*

(Applicable to free acid)

(a) Dissolve 0.25 g of sample in ca 10 ml of H<sub>2</sub>O and add a few drops of 10% FeCl<sub>3</sub> soln. A bright yellow color is produced. This is a general test for hydroxy acids and is not specific for mandelic acid.

(b) Dissolve 0.25 g of sample in 5 ml of H<sub>2</sub>O in test tube; to soln add 5 ml of H<sub>2</sub>SO<sub>4</sub> and agitate test tube and contents a few seconds; then add 10 ml of H<sub>2</sub>SO<sub>4</sub>, so as to form two layers. Agitate very gently but do not mix. Purple color slowly forms at interface if test tube is allowed to stand for a few minutes. A strong odor of benzaldehyde is noticed on shaking.

## 39.159

## DETERMINATION

(a) *Tablets.*—Weigh a quantity of powdered material equivalent to 0.4–0.5 g of mandelic acid and transfer to separator containing 10 ml of H<sub>2</sub>O. Acidify with HCl (1+3) and add 2 ml in excess. Extract with six 20 ml portions of CHCl<sub>3</sub>-ether solvent (2+1); wash each portion in second separator with 2 ml of H<sub>2</sub>O, and pass soln thru plug of cotton, previously saturated with solvent, into 250 ml beaker. Wash outer surface of stem of separator with a few ml of solvent and add this to main portion. Test for complete extraction with 15 ml more of solvent and evaporate in separate beaker. Wash any residue thus obtained into beaker containing main extract with a few ml of solvent. Evaporate to dryness at temp. not exceeding 40° with aid of fan. Dissolve residue in 25 ml of CO<sub>2</sub>-free H<sub>2</sub>O and titrate with 0.1 N NaOH, using phenolphthalein as indicator. 1 ml of 0.1 N NaOH = 0.01521 g of mandelic acid (C<sub>6</sub>H<sub>5</sub>CHOHCOOH), 0.01691 g of NH<sub>4</sub> mandelate (C<sub>6</sub>H<sub>5</sub>CHOHCOONH<sub>4</sub>),

0.01741 g of Na mandelate ( $C_6H_5CHOHCOONa$ ), 0.01711 g of Ca mandelate ( $(C_6H_5CHOHCOO)_2Ca$ ), or 0.01632 g of Mg mandelate ( $(C_6H_5CHOHCOO)_2Mg$ ).

After titration the mandelic acid may be re-extracted and extract used for melting point determinations or qualitative tests.

(b) *Liquid preparations*.—Measure 1 ml of sample or an aliquot of a dilution sufficient to yield 0.4–0.5 g of mandelic acid into separator and acidify with HCl (1+3). Proceed as directed in (a).

### 39.160 NICOTINIC ACID IN TABLETS AND AMPULS—See 36.46

#### METHYL ALCOHOL (0.5–5.0%) IN PRESENCE OF ETHYL ALCOHOL (80)—TENTATIVE

### 39.161

#### REAGENTS

(a) *Soln A*.—Methyl alcohol, 25% by volume ( $\pm 0.1\%$ ).

(b) *Soln B*.—Mix 20 ml of Soln A and 95 ml of absolute ethyl alcohol (or equivalent in dilute alcohol) with  $H_2O$  to volume of 2 liters. Make all transfers and dilutions at  $20^\circ$ .

(c) *Fuchsin-sulfurous acid*.—Dissolve 0.2 g of fuchsin in 120 ml of hot  $H_2O$ , cool soln, and add 2 g of  $Na_2SO_3$  in 20 ml of  $H_2O$ . Mix, add 2 ml of HCl, and dilute to 200 ml.

### 39.162

#### DETERMINATION

(a) *Total alcohols*.—Measure at room temp. ( $20^\circ$ ) 25 ml of sample, add 90 ml of  $H_2O$ , neutralize to litmus with 5% NaOH soln, distil, and dilute volume of distillate to 100 ml at same temp. as noted when original aliquot was measured. Determine total alcohol (as ethyl alcohol) from the sp. gr. of distillate in usual way and estimate percentage of alcohol in original soln by means of proper dilution factor. Test a portion of this distillate by U.S.P. test for methyl alcohol, taking precaution to determine that HCHO, as such, is not present. If methyl alcohol is present, transfer 10 ml of distillate to separator, add 40 ml of saturated NaCl soln, shake with 25 ml of petroleum benzene, and draw off the aqueous NaCl soln into distilling flask. Wash the petroleum benzene in the separator with two 10 ml portions of saturated NaCl soln, adding these to portion already in distilling flask. Distil, receiving distillate in 50 ml graduated flask. Calculate quantity of ethyl alcohol to add to this distillate to make a 5% soln of total alcohol (assuming it to be all ethyl alcohol) when made up to 50 ml, add this calculated amount, and make to volume of 50 ml. Transfer 5 ml of this distillate to 200 ml volumetric flask for color comparison with standards.

(b) *Color standards*.—Transfer to 200 ml volumetric flasks a series of aliquots, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 ml of Soln B, adding 4.5, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.5, and 0 ml, respectively, of 5% ethyl alcohol. (These amounts of methyl alcohol represent percentages in original unknown soln when unknown is diluted as outlined above.)

(c) *Methyl alcohol*.—To each of the standards and to the unknown, add 1 ml of  $H_3PO_4$  (1+1) and 2 ml of 3%  $KMnO_4$  soln, and allow mixtures to stand 10 min. Add 1 ml of 10% oxalic acid soln and allow mixtures to stand until clear or transparent. Add 5 ml of  $H_2SO_4$  soln (1+3) and 5 ml of the freshly prepared fuchsin- $H_2SO_4$  soln and allow solns to stand  $1\frac{1}{2}$  hours. Dilute to 200 ml, mix thoroly, and transfer equal quantities to series of test tubes of uniform color and diameter for color comparison. Compare unknown with standard which it most nearly approaches in color intensity, approximating intervals less than 0.5%, if desired. Value obtained represents percentage of methyl alcohol in original sample.

(d) *Ethyl alcohol*.—Deduct percentage of methyl alcohol, determined colorimetrically, from percentage of total alcohols previously determined.

## ACETONE AND ISOPROPYL AND ETHYL ALCOHOLS—QUALITATIVE TESTS (81)—TENTATIVE

*Acetone (in absence of other ketones)*

39.163

## REAGENT

*2,4-dinitrophenylhydrazine soln.*—Suspend 2 g of 2,4-dinitrophenylhydrazine in 15 ml of 2 *N* HCl, add 10 ml of HCl, then 600 ml of 2 *N* HCl, and filter.

39.164

## TEST

To 1 ml of sample add 5 ml of the reagent; acetone gives a precipitate. To obtain sufficient material for identification, treat 5 ml of sample with 50 ml of reagent. After swirling mixture and allowing to stand for 15 min., filter into Gooch crucible and dry at 100°. Dissolve the hydrazone in hot alcohol, and filter; concentrate filtrate to ca 5 ml and allow to cool and crystallize. Acetone 2,4-dinitrophenylhydrazone melts at 128°.

*Isopropyl and Ethyl Alcohols*

39.165

## REAGENTS

(a) *3,5-dinitrobenzoyl chloride.*—The c.p. crystalline material obtained from Eastman Kodak Company can be used without further purification.

(b) *Ether-petroleum benzine solvent.*—Mix 1 volume of ether with 5 volumes of petroleum benzine. The 3,5-dinitrobenzoates are easily soluble in this solvent whereas 3,5-dinitrobenzoic acid is practically insoluble.

(c) *Lanthanum nitrate soln.*—Dissolve 0.5 g of  $\text{La}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$  in 10 ml of  $\text{H}_2\text{O}$ .

## TEST

39.166

*I. When either is present in at least 5% aqueous solution*

Transfer 100 ml of sample to 150 ml Claisen flask fitted with rather long side arm bent downward at a right angle ca 4" from end. (Test tube immersed in ice-NaCl mixture is convenient receiver.) Place a bumping stone in flask, fitted with a thermometer, and immerse flask in beaker of  $\text{H}_2\text{O}$ . Heat  $\text{H}_2\text{O}$  to boiling and continue distillation until thermometer inside flask reaches 90°. Add to receiver ca 1 g of  $\text{Na}_2\text{SO}_4$  and keep in refrigerator 1–2 hours to complete drying of the alcohol. Filter thru small funnel into second test tube containing ca 0.1 g of 3,5-dinitrobenzoyl chloride. Stopper tube immediately and immerse lower end in  $\text{H}_2\text{O}$  at 75–80°. Shake gently and continue heating for 30 min. Cool soln, scratching side of tube to induce crystallization. Extract crystalline material with the ether-petroleum benzine by filling tube with solvent and shaking. Filter into separator. Repeat extraction 4 or 5 times. Extract ether soln with 5%  $\text{Na}_2\text{CO}_3$  soln, wash thoroly with  $\text{H}_2\text{O}$ , and filter thru funnel containing  $\text{Na}_2\text{SO}_4$ . Collect filtrate in beaker, evaporate solvent, and determine melting point of residue.

Isopropyl 3,5-dinitrobenzoate melts at 122°. Ethyl 3,5-dinitrobenzoate melts at 92°.

39.167

*II. When either or both are present in approximately 1% aqueous solution*

Transfer ca 10 ml of sample to 50 ml Erlenmeyer flask and add 10 ml of the dinitrophenylhydrazine reagent, 39.163. Formation of flocculent precipitate of acetone 2,4-dinitrophenylhydrazone indicates presence of acetone. If acetone is present, proceed as directed in (a), and if absent, proceed as directed in (b).

(a) *Acetone present.*—Place 10 ml of sample in 200 ml Erlenmeyer flask and add 10 ml of  $\text{H}_2\text{O}$ , 0.4–0.5 g of paraformaldehyde, and 10 ml of 5% NaOH soln. Place flask on hot plate under reflux and heat to boiling. Continue the heating to formation

of a resin as indicated by appearance of precipitate. While mixture is still hot add 50 ml of Fehling soln thru top of condenser. (Excess should be present, indicated by characteristic blue color.) Allow mixture to cool, transfer to 500 ml Kjeldahl flask, and distil into another Kjeldahl flask until ca 50 ml is collected. To distillate add 50 ml of 10%  $K_2Cr_2O_7$  soln, followed by 100 ml of  $H_2SO_4$  (1+3). Let mixture stand with occasional swirling for 1 hour, then add 100 ml of 25%  $FeSO_4 \cdot 7H_2O$  soln and distil slowly. When 25 ml of distillate is collected change receivers and collect an additional 50 ml. Test first fraction with the dinitrophenylhydrazine reagent; precipitate indicates presence of isopropyl alcohol. Test ca 5 ml of second fraction for acetic acid by adding 1 ml of the  $La(NO_3)_3$  soln, followed by 1 ml of 0.02 *N* I and 1 drop of  $NH_4OH$ . Heat over burner, and if acetic acid is present (indicating ethyl alcohol) a deep blue color will develop.

(b) *Acetone absent*.—Transfer 10 ml of sample to 50 ml Erlenmeyer flask and add 10 ml of  $HgSO_4$  reagent, 16.20. Place on steam bath and heat for 15 min. White-yellow precipitate indicates presence of isopropyl alcohol. If it is present, transfer 10 ml of sample to Kjeldahl flask, add 50 ml of  $H_2O$ , and proceed as directed in (a), beginning "add 50 ml of 10%  $K_2Cr_2O_7$ . . . ."

If isopropyl alcohol is absent, test 10 ml of sample by adding a few drops of 5%  $NaOH$  soln and an excess of I-KI soln. Odor of iodoform indicates presence of ethyl alcohol.

#### PHENOLPHTHALEIN IN CHOCOLATE PREPARATIONS (82)—OFFICIAL

##### 39.168

##### REAGENTS

(a) *Iodine soln*.—Dissolve 20 g of KI in minimum quantity of  $H_2O$ , add 14 g of I, and when dissolved dilute to 120 ml. Add sufficient KOH soln (1+1) to discharge the I color.

(b) *Sodium sulfite soln*.—Dissolve 15 g of  $Na_2SO_3$  in  $H_2O$  and dilute to 100 ml.

##### 39.169

##### PREPARATION OF ALCOHOLIC EXTRACT

To 1 g sample in 50 ml volumetric flask, add ca 35 ml of alcohol; boil gently ca 20 min., rotating flask occasionally; cool, and make to volume with alcohol. Mix thoroly, filter thru dry paper (covering funnel with watch-glass to avoid evaporation), pipet a number of aliquots of 10 ml each (or sufficient to contain ca 0.2 g of phenolphthalein) into 250 ml beakers, and evaporate to dryness on steam bath.

##### 39.170

##### DETERMINATION

Take up residue in alkali by moistening with ca 1 ml of KOH soln (1+1) and add a little  $H_2O$ . When residue is completely in soln add a piece of ice (ca 40 g), 4–4.5 ml of the prepared I reagent, and HCl from a buret, dropwise, using stirring rod (beaker is not rotated), to complete precipitation. If sufficient I has been added, precipitate as well as supernatant liquid will be brown; if not, add more I to insure excess, and then the KOH soln dropwise, with stirring, to dissolve precipitate completely and consume all excess I. (This soln should be blue or blue-purple.) Repeat process of precipitation with strong acid and resolution with strong alkali 3 or 4 times with small quantities of the reagents, adding small pieces of ice if necessary to keep soln cold. In the acid condition there should be a brown precipitate resembling a periodide, and supernatant liquid should be colored brown by the excess I. (The alkaline soln should be clear blue or purple-blue, and no precipitate should be present.) Then add 1 or 2 ml of the  $Na_2SO_3$  soln to the alkaline soln and filter the ice-cold mixture thru a Gooch crucible into tall 250 ml beaker, using bell jar arrangement and washing several times with  $H_2O$ . Acidify filtrate with HCl, using a few ml in

excess, and heat on steam bath 20–30 min. Collect coagulated precipitate in weighed Gooch crucible, wash a few times with  $H_2O$ , and when sucked fairly dry wash several times with petroleum benzine. Dry precipitate in oven ( $120$ – $140^\circ$ ) to constant weight. Weight of precipitate  $\times 0.3872$  = weight of phenolphthalein.

#### PHENOLPHTHALEIN IN EMULSIONS (83)—TENTATIVE

##### 39.171

##### REAGENT

*Solvent mixture.*—Mix 25 ml of alcohol with 75 ml of ether.

##### 39.172

##### DETERMINATION

Shake the sample well (preferably in mechanical shaker, for 10 min.). Weigh accurately a quantity of sample equivalent to ca 0.1 g of phenolphthalein from weighing buret directly into a centrifuge bottle. Add 100 ml of the solvent mixture, stopper bottle, shake vigorously, and then centrifuge until clear. Decant into separator. Wash residue in bottle twice with 10 ml portions of the solvent mixture, adding these washings to separator. Dissolve residue in bottle in a few ml of  $H_2O$  and reprecipitate gums with 50 ml of the solvent mixture. Again shake and centrifuge as before, decanting into separator. Wash residue and bottle with three 10 ml portions of the solvent mixture and add these to separator. Dissolve residue in a few ml of  $H_2O$  and test for complete extraction with NaOH.

Shake the solvent mixture in separator repeatedly with 25 ml portions of ca 0.1  $N$  NaOH until the phenolphthalein has been completely removed as shown by absence of color. Combine alkaline extracts in another separator and acidify solution with dilute  $H_2SO_4$ .

Extract the phenolphthalein by shaking the acid mixture repeatedly with 10 ml portions of ether. Test for complete extraction with NaOH soln. Combine the ether extracts in a 150 ml beaker, evaporate to dryness, and determine the phenolphthalein as directed in 39.170, omitting the operation, "filter the ice-cold mixture thru a Gooch . . . washing several times with  $H_2O$ ."

#### PHENOLPHTHALEIN IN TABLETS

##### *Ether Extraction Method (82)—Official*

(Not applicable in presence of other ether extractives)

##### 39.173

##### PREPARATION OF SAMPLE—See 39.1

##### 39.174

##### DETERMINATION

Weigh portion of powdered material representing ca 0.2 g of phenolphthalein, transfer to separator by means of 10 ml of 5% NaOH soln and a little  $H_2O$ , and extract 3 or 4 times with ether, using 25 ml for the first and 20 ml for each subsequent extraction. Transfer ether extracts to second separator and wash twice with 5 ml of the 5% NaOH soln. (Substances like quinine, acetanilid, acetophenetidin as well as any unsaponified fatty material or mineral oil, if present, will be removed by extraction with ether.) Combine alkaline solns and acidify with HCl. Extract with ether as before, until all the phenolphthalein has been removed, as determined by testing a portion of ether soln with NaOH (after 4 or 5 extractions). Filter ether extractions into weighed beaker, evaporate, dry residue at  $100^\circ$ , and weigh. Residue should be soluble in alcohol, showing absence of most oils. If titrated with 0.1  $N$  NaOH, alcoholic soln should be practically neutral, showing absence of acid extractives such as fatty acids and salicylic or benzoic acid.

## PHENOTHIAZINE (84)—TENTATIVE

39.175

## REAGENTS

(a) *Standard phenothiazine*.—Prepare a 1+10 toluene soln of phenothiazine with aid of heat. Add 0.1 g of activated charcoal for each 4 g of phenothiazine. Boil 10 min. under reflux and filter while hot thru heated filter. Cool soln and collect phenothiazine crystals on suction filter. Dry crystals in oven at 100° and then in vacuum desiccator containing paraffin chips. Repeat recrystallization process, if necessary, until product melts at 184–185°.

(b) *Bromine water*.—Saturated aqueous soln.

39.176

## APPARATUS

*Photoelectric colorimeter*.—Any of the various types of photoelectric colorimeters is satisfactory. Visual colorimeter may be substituted but accuracy is decreased.

39.177

## DETERMINATION

Calibrate the photoelectric colorimeter by following procedure: Weigh quantities (90–110 mg) of standard phenothiazine, develop color as described below, obtain transmission readings with 500–530 m $\mu$  filter, and plot mg of phenothiazine against colorimeter readings.

Weigh accurately quantity of sample containing ca 100 mg ( $\pm 10\%$ ) of phenothiazine and transfer to 300–500 ml glass-stoppered bottle. Add from pipet 200 ml of alcohol, stopper bottle, and shake until phenothiazine is completely dissolved. Pipet 5 ml of clear, supernatant soln into 100 ml glass-stoppered volumetric flask. Add 45 ml of alcohol and heat 10 min. in water bath at 60°. Add rapidly from graduated cylinder 5 ml of the Br-H<sub>2</sub>O, stopper tightly, and heat 15 min. in water bath at 60°. Add an additional 5 ml of Br-H<sub>2</sub>O, stopper tightly, and allow to stand 10 min. outside the bath. Return open flasks to water bath, heat bath to ca 90°, and continue heating 5 min. after alcohol vapors begin to escape from flask (to remove excess Br). Cool to room temp. and dilute to mark with alcohol. Examine colored soln in colorimeter and compare colorimeter reading with standard curve. (Results of duplicate determinations should agree within  $\pm 0.4\%$ , and analyses by different analysts should agree within  $\pm 2\%$ .)

## PYRIDIDIUM (85)—OFFICIAL, FIRST ACTION

39.178

## REAGENTS

(a) *Standard titanium trichloride soln*.—Prepare as directed in 21.35, and standardize as directed in 21.36, Method II.

(b) *Light green S F yellowish soln*.—Dissolve 1 g in H<sub>2</sub>O and dilute to 1000 ml.

39.179

## PREPARATION OF SAMPLE

(a) *Solutions*.—To volume containing ca 0.1 g of pyridium, add 10 ml of 0.1 N HCl and dilute to 100 ml.

(b) *Tablets and jelly*.—Weigh quantity of sample (powdered in case of tablets) equivalent to ca 0.1 g of pyridium, add 10 ml of 0.1 N HCl, and dilute to 100 ml.

(c) *Ointments*.—Weigh in 100 ml beaker a portion of sample equivalent to ca 0.1 g of pyridium, stir with ether until ointment base is dissolved, and wash into separator with ether and H<sub>2</sub>O. Shake thoroly and draw off aqueous layer into second separator containing 25 ml of ether. Shake, and draw off aqueous layer into third separator containing 25 ml of ether. Shake, and transfer aqueous layer to 250 ml beaker. Wash

ether layers with alternate 10 ml portions of HCl (1+1) and H<sub>2</sub>O until no more color is removed, passing each portion of HCl or H<sub>2</sub>O successively thru the three separators and finally into beaker. Nearly neutralize combined acid extracts with NH<sub>4</sub>OH, cool, wash into separator, make ammoniacal, and extract with 25 ml portions of CHCl<sub>3</sub> until no more color is removed, filtering the CHCl<sub>3</sub> thru pledget of cotton in stem of separator. Evaporate combined CHCl<sub>3</sub> extracts just to dryness, take up in 10 ml of 0.1 N HCl, and dilute to 100 ml.

## 39.180

## DETERMINATION

Heat soln to boiling, add 15 g of Na acid tartrate, and boil 2 min. Add 10 ml of the light green S F yellowish soln and titrate hot with the standard TiCl<sub>3</sub> soln in a current of CO<sub>2</sub>. End point is the change from green to pale yellow. Run a blank titration with 10 ml of 0.1 N HCl, 90 ml of H<sub>2</sub>O, 15 g of Na acid tartrate, and 10 ml of the light green SF yellowish soln, and subtract from volume of TiCl<sub>3</sub> previously found. 1 ml of 0.1 N TiCl<sub>3</sub> = 0.00624 g of pyridium (C<sub>11</sub>H<sub>11</sub>N<sub>5</sub>.HCl).

## QUINACRINE (88)—TENTATIVE

## 39.181

## APPARATUS

(a) *Spectrophotometer or photoelectric photometer.*—Having filter with peak transmittance at 425 mμ.

(b) *Matched 1 cm absorption cells.*

## 39.182

## REAGENTS

(a) *Hydrochloric acid.*—Approximately 0.1 N.

(b) *Standard quinacrine hydrochloride soln.*—Determine purity of a sample of the salt, either by assay by the U.S.P. method (U.S.P. XII, p. 383) or by determining N according to 2.24. Using purity figure so obtained, prepare a soln containing 2.5 mg of quinacrine hydrochloride in 100 ml of 0.1 N HCl.

## 39.183

## DETERMINATION

Accurately weigh or measure a quantity of sample containing ca 100 mg of quinacrine hydrochloride and transfer to liter volumetric flask. Add ca 100 ml of the 0.1 N HCl and heat on steam bath until the quinacrine hydrochloride has dissolved. Cool, and dilute to volume with the HCl.

Filter soln if not perfectly clear. Pipet an aliquot containing 2–3 mg of quinacrine hydrochloride into 100 ml volumetric flask and fill to mark with the 0.1 N HCl. Determine the absorption (E) of this soln and also of the standard soln, relative to a blank of 0.1 N HCl at 425 mμ.

$$\text{Mg of quinacrine hydrochloride in aliquot} = \frac{E \text{ sample}}{E \text{ standard}} \times 2.5.$$

## QUININE (SPECTROPHOTOMETRIC METHOD) (87)—TENTATIVE

## 39.184

## APPARATUS

A spectrophotometer suitable for measuring absorption in the ultraviolet. A slit that isolates a portion of the spectrum 5 mμ or less is desirable. Two matched 1 cm absorption cells.

## 39.185

## REAGENTS

(a) *Hydrochloric acid.*—Approximately 0.1 N.

(b) *Standard quinine soln.*—Recrystallize quinine N.F. VII twice from benzene and dry to constant weight at 100°. Prepare a soln containing 5.0 mg of this (anhydrous) quinine in 100 ml of 0.1 *N* HCl. (Keeps indefinitely.)

## 39.186

## DETERMINATION

(a) *In presence of those compounds having no absorption in HCl soln at 347.5 mμ.* (These compounds include strychnine, atropine, ephedrine, caffeine, acetylsalicylic acid, acetanilid, acetophenetidin, camphor, phenolphthalein, glycerol, alcohols; most green, blue, and red dyes; and sugars; all of which are frequently found in preparations containing quinine.)—Accurately weigh or measure quantity of sample containing ca 0.1 g of quinine and transfer to liter volumetric flask. Dissolve in 25 ml of HCl (1+4) and make to volume so as to have final concentration ca 0.1 *N* in HCl. Filter soln if not perfectly clear. Pipet an aliquot containing 2–5 mg of quinine into 100 ml volumetric flask and fill to mark with 0.1 *N* HCl. Determine absorption (*E*) relative to a blank of 0.1 *N* HCl at 347.5 mμ, also determine the absorption (*E*) of the standard soln relative to the same blank.

$$\text{Mg quinine (anhydrous) in aliquot} = \frac{E \text{ sample}}{E \text{ standard}} \times 5.0.$$

(b) *Applicable in presence of ferric compounds, such as elixir of iron, quinine, and strychnine.*—Proceed as directed in (a), but add 10 ml of H<sub>3</sub>PO<sub>4</sub> to the soln in a 100 ml flask before making to volume. Standard soln should contain 10 ml of H<sub>3</sub>PO<sub>4</sub>/100 ml and blanks should consist of 0.1 *N* HCl containing 10 ml of H<sub>3</sub>PO<sub>4</sub>/100 ml.

(c) *Applicable in presence of interfering substances.*—Before determining quinine by its absorption at 347.5 mμ, separate it from the following compounds: Aloin, podophyllin, anthraquinone derivatives, other cinchona alkaloids, and yellow dyes. These absorb light in the region of 347.5 mμ.

## 39.187

## SULFANILAMIDE (88)—OFFICIAL

Place on a 9 cm folded filter paper in a funnel a portion of sample containing ca 0.5 g of sulfanilamide. Wash soluble portion with fine stream of acetone into 250 ml flask, using total of ca 25 ml of acetone. Test for complete extraction by evaporating small portion of washings. Immerse flask in water bath at ca 70° until acetone has been evaporated and its odor is no longer perceptible. Remove from bath and add 10–12 ml of 75% (by volume) H<sub>2</sub>SO<sub>4</sub>. Connect flask to reflux condenser with water jacket, add a few glass beads, and boil soln slowly 30 min. Wash down condenser with H<sub>2</sub>O, make liquid in flask to ca 100 ml with H<sub>2</sub>O, add an excess of 50% alkali, distil, and collect the NH<sub>3</sub> in distillate in an excess of 0.1 *N* H<sub>2</sub>SO<sub>4</sub>. Titrate excess acid with 0.1 *N* NaOH, using methyl red indicator. 1 ml of 0.1 *N* H<sub>2</sub>SO<sub>4</sub> = 0.01722 g of (NH<sub>2</sub>)<sub>2</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>2</sub>.

## SANTONIN IN MIXTURES

## 39.188

*Langer Method (Modified) (89)—Official*

Weigh out a sample equivalent to ca 0.15 g of santonin, and extract with 10, 10, 10, 5, and 5 ml portions of petroleum benzine saturated with santonin. (If sample is fat-free this step may be omitted.) Filter each portion of solvent with aid of suction to complete dryness thru a Gooch crucible provided with asbestos mat before following with another portion of fresh solvent. Extract residue in soln flask and crucible with 15, 10, 5, and 5 ml of hot benzene, filtering each portion as before. Evaporate the benzene extract in tared flask and dry residue to constant weight at 100°. Weight of santonin in flask = weight of santonin in sample.



*Dinitrophenylhydrazine Method (90)—Official*

## 39.189

## REAGENT

*Dinitrophenylhydrazine sulfate soln.*—Dissolve 1 g of 2,4 dinitrophenylhydrazine in a mixture of 90 ml of  $H_2O$  and 10 ml of  $H_2SO_4$  by warming, cool, and filter.

## 39.190

## DETERMINATION

Weigh 2.5 g of ground sample into Gooch crucible and wash with ca 100 ml of petroleum benzene saturated with santonin. Discard washings. Extract with ca 100 ml of hot benzene, collecting filtrate in beaker. Evaporate to dryness, warm residue with alcohol until dissolved, transfer to 100 ml volumetric flask, cool, make to volume at  $20^\circ$  with alcohol, and filter if necessary. To 25 ml of the soln add 50 ml of the dinitrophenylhydrazine soln and allow to stand 48 hours in dark place. Collect precipitate in Gooch crucible and wash with dilute alcohol (1+2), using a total volume of ca 150 ml. Dry residue 1 hour at  $100^\circ$ , cool, and weigh. Weight of precipitate  $\times 0.5775$  = weight of santonin.

## 39.191 SANTONIN IN SANTONICA (LEVANT WORM SEED) (91)—TENTATIVE

Extract 3 g of ground sample with benzene in Soxhlet apparatus or automatic percolator (Fig. 63) for 3 hours. Wash extract into a separator with a little benzene, add more benzene if necessary to make total volume of ca 100 ml, and shake vigorously for 5 min. with 35 ml of 8%  $Na_2CO_3$  soln. Allow mixture to separate completely and transfer aqueous layer to second separator. Wash the benzene once with 10 ml of  $H_2O$  and add washing to second separator. Shake combined aqueous extracts with 10 ml of benzene, discard aqueous layer, wash benzene with 5 ml of  $H_2O$ , and combine with benzene in first separator. Filter the benzene soln thru cotton and evaporate filtrate to dryness. Warm residue with 5 ml of alcohol until mass is disintegrated, and add 60 ml of saturated aqueous  $Ba(OH)_2$  soln while stirring. Heat mixture to boiling, place on steam bath 10 min., filter into a separator, and wash filter and beaker with two 10 ml portions of hot  $Ba(OH)_2$  soln. Add 6 ml of  $HCl$  (2+1) to filtrate, cool, and extract with 25, 15, 10, 10, and 5 ml portions of  $CHCl_3$ , filtering thru pledget of cotton in stem of funnel, and evaporate filtrate to dryness. Dissolve residue in 25 ml of alcohol by warming, mix soln with 50 ml of dinitrophenylhydrazine sulfate soln, 39.189, and proceed as directed in 39.190, beginning "allow to stand 48 hours."

## ARSENIC IN IRON-ARSENIC TABLETS (92)—OFFICIAL

## 39.192

## REAGENT

*Standard soln of potassium bromate (or of iodine).*—Standardize against pure  $As_2O_3$ . (The strength of this soln is a matter of choice. 0.5625 g of  $KBrO_3$  dissolved in  $H_2O$  and diluted to 1 liter gives a soln that is 0.02021 N, 1 ml of which = 0.001 g of  $As_2O_3$ .)

## 39.193

## APPARATUS

Use either the Ramberg-Sjöström As flask, which consists of a 300 ml Kjeldahl flask provided with a specially shaped outlet tube connected with the flask by means of a ground joint (A, Fig. 67), or a 300 ml Kjeldahl flask provided with an outlet tube, internal diameter of main part of which is ca 13 mm and that of contracted tip ca 5 mm, connected with the flask by means of a rubber stopper (B).

## 39.194

## DETERMINATION

Weigh and place in the flask 5–10 tablets or pills, add 10–15 ml of  $H_2O$ , and allow to soak 30 min. Add, small portions at a time, 20 ml of fuming  $HNO_3$ , cooling if necessary to prevent loss by frothing. When reaction has ceased, add carefully and in small portions at a time 25–28 ml of  $H_2SO_4$ . Place flask in inclined position on asbestos mat and heat over small flame. As soon as greater part of the  $HNO_3$  has been driven off, and while still heating, drop in 8 ml of fuming  $HNO_3$  thru a suitably placed thistle tube and heat over larger flame until  $SO_3$  is evolved. If after cooling

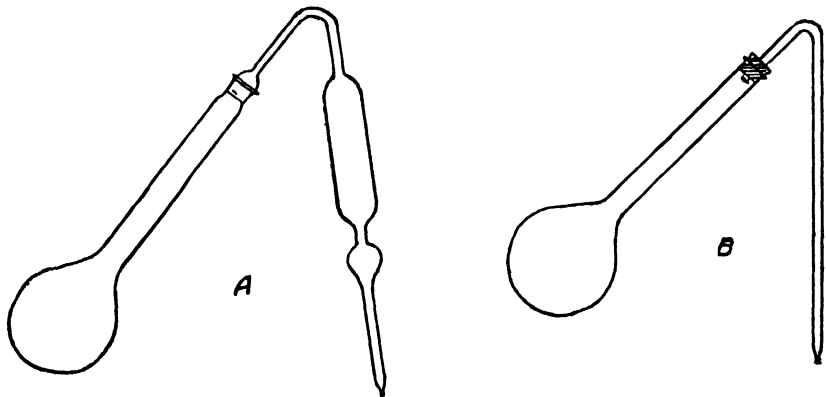


FIG. 67.—APPARATUS FOR DETERMINATION OF ARSENIC IN IRON-ARSENIC TABLETS

the precipitated sulfates are not colorless or pale yellow and are not free from gray or black particles, heat contents of flask further with an additional 10 ml of fuming  $HNO_3$ . (It is essential that all organic matter be destroyed.) To cooled mixture add 30 ml of saturated  $NH_4$  oxalate soln; heat until fumes of  $SO_3$  are evolved and, to insure complete destruction of the oxalic acid, for 10 min. thereafter over low flame; cool; and add 20 ml of  $H_2O$  while gently swirling flask. Dry neck of flask over small flame and add 30 g of  $NaCl$ , 5 g of  $FeSO_4 \cdot 7H_2O$  (or 1 g of  $N_2H_4 \cdot H_2SO_4$ ), 1 g of  $NaBr$ , and 25 ml of  $HCl$ . Mix contents of flask and connect delivery tube. If the Ramberg-Sjöström apparatus is used, moisten the ground-glass joint with a drop of  $H_2SO_4$ . Fix flask in inclined position with tip of outlet tube ca 1 cm under surface of 150 ml of  $H_2O$  in an Erlenmeyer flask surrounded by ice or by cold  $H_2O$ . Distil at such a rate that bend at top of tube becomes warm in 4 min. and lower end in ca 8 min. from time heat is applied. Discontinue distillation at end of 10 min., but before removing flame lift distillation flask until tip of outlet tube is above the  $H_2O$  in the receiving flask. Let outlet tube drain, remove receiver, and either titrate with the standard  $KBrO_3$  soln, using 2 drops of methyl orange indicator (red color of indicator at end point may fade slowly, but color should persist at least 1 min. upon addition of another drop of indicator); or nearly neutralize with  $NaOH$ , add 4–5 g of  $NaHCO_3$ , and titrate with the standard  $I$  soln, using starch indicator, 6.3(e).

## 39.195

## ARSENIC IN SODIUM CACODYLATE (ss)—OFFICIAL

Transfer 0.2 g of sample, accurately weighed, to Kjeldahl flask. Add 10 g of  $K_2SO_4$ , 0.3 g of starch, and 20 ml of  $H_2SO_4$ . Digest over low flame until frothing has

ceased. Continue digestion 4 hours or until mixture is colorless. Cool, dilute with  $\text{H}_2\text{O}$ , and transfer to 500 ml Erlenmeyer flask. Add  $\text{NaOH}$  soln (1+1) slowly until alkaline to litmus paper, and acidify with  $\text{H}_2\text{SO}_4$ . Place flask in  $\text{H}_2\text{O}$  until thoroly cooled, add 5 g of  $\text{NaHCO}_3$ , and titrate with 0.1 *N*  $\text{I}$ . Conduct a blank, using same quantities of reagents. 1 ml of 0.1 *N*  $\text{I}$  = 0.00375 g of  $\text{As}$ , or 0.00800 g of anhydrous  $\text{Na}(\text{CH}_3)_2\text{AsO}_2$ .

## 39.196

## ARSENIC IN IRON METHYLARSENATE (94)—OFFICIAL

Transfer a suitable quantity of sample (0.2 g, if practicable) to a Kjeldahl flask. Add 10 g of  $\text{K}_2\text{SO}_4$ , 0.3 g of starch, and 20 ml of  $\text{H}_2\text{SO}_4$ . Digest over low heat until frothing has ceased and continue digestion over slightly higher flame until mixture is colorless. Cool, and add 20 ml of  $\text{H}_2\text{O}$ . Dry neck of flask over small flame; cool contents; and add 30 g of  $\text{NaCl}$ , 5 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 1 g of  $\text{NaBr}$ , and 25 ml of  $\text{HCl}$ . Distil as directed under 39.194. Conduct a blank, using same quantities of reagents.

## 39.197

## BISMUTH COMPOUNDS IN TABLETS (95)—OFFICIAL

(Lead absent)

Thoroly mix sample and weigh 0.5 g into 500 ml Kjeldahl flask. Ignite gently over small flame, using wire gauze under flask, and increase heat towards end. Allow to cool, add 15–20 ml of  $\text{HNO}_3$ , evaporate to dryness, and ignite as before until yellow or orange  $\text{Bi}_2\text{O}_3$  is formed. Cool residue, and dissolve in 10–15 ml of warm  $\text{HNO}_3$ , using a few ml of 3%  $\text{H}_2\text{O}_2$  if there is difficulty in obtaining a soln. Boil off excess  $\text{H}_2\text{O}_2$ , and wash into 400 ml beaker with  $\text{H}_2\text{O}$ , rinsing flask well. Dilute to ca 200 ml, make just neutral to litmus with  $\text{NH}_4\text{OH}$ , and add 5 ml of  $\text{HCl}$ . Precipitate with  $\text{H}_2\text{S}$  completely.

Transfer precipitate to a filter paper and wash once with  $\text{HCl}$  (5+200) and then several times with  $\text{H}_2\text{O}$ . Dissolve the precipitate of  $\text{Bi}_2\text{S}_3$  on filter with hot  $\text{HNO}_3$  (1+2). A small residue of  $\text{S}$  (and  $\text{HgS}$  if  $\text{Hg}$  salts are present) usually remains. Neutralize filtrate with 10%  $\text{NH}_4\text{OH}$  and precipitate with 25 ml of 20%  $(\text{NH}_4)_2\text{CO}_3$ . Concentrate to ca 150 ml (by boiling, if desired) and allow to stand on steam bath 1–2 hours. Collect precipitate in previously ignited weighed Gooch crucible, wash with small quantity of  $\text{H}_2\text{O}$ , dry, ignite in muffle at red heat, and weigh as  $\text{Bi}_2\text{O}_3$ .

## 39.198

## CALCIUM GLUCONATE (96)—OFFICIAL

(Applicable to preparations the aqueous solns of which are neutral and do not contain salts of other optically active hydroxy acids)

Weigh two 0.5 g portions of  $\text{Ca}$  gluconate or two 1 g portions of powdered tablets containing 50% or less of the salt. If chocolate or a fatty base is present, wash samples several times on hardened filter with absolute ether and warm residue until ether is driven off. Transfer each portion to a separate 25 ml volumetric flask, add 15 ml of  $\text{H}_2\text{O}$ , and warm until the  $\text{Ca}$  salt is dissolved (there will be an undissolved residue in the case of samples containing cocoa). Cool mixture to room temp. To one flask (No. 1) add 3.5 g of finely pulverized uranyl acetate, stopper, and place mixture in shaking machine for 1 hour (if agitation is not sufficiently vigorous, more than 1 hour's shaking may be required). Allow other flask (No. 2) to stand. If sample contains chocolate, add a little alumina cream, 34.19(b), to each flask. Cool to 20°, make up contents of flask No. 1 to volume with uranyl acetate soln (10 g shaken with 95 ml of  $\text{H}_2\text{O}$  until saturated, then filtered), and flask No. 2 with  $\text{H}_2\text{O}$ . Filter, and polarize each soln in a 200 mm tube, using a 50 mm tube containing 1.8%  $\text{K}_2\text{Cr}_2\text{O}_7$  soln as a light filter. If soln is too dark to read in the 200 mm tube, make

reading in a 100 mm tube and multiply result by 2. If  $A$  = rotation in °V. of soln No. 2 and  $B$  = the rotation of soln No. 1, with 1 g samples the percentage of  $\text{Ca}(\text{C}_6\text{H}_{11}\text{O}_7)_2$  =  $4.34 (B - A)$ , and with 0.5 g samples the percentage of  $\text{Ca}(\text{C}_6\text{H}_{11}\text{O}_7)_2$  =  $8.52 (B - A)$ .

#### HYPOPHOSPHITES IN SIRUPS—OFFICIAL

(Applicable in absence of phosphates; if phosphates are present, make suitable correction)

#### 39.199

##### Method I (97)

(a) *Total hypophosphites*.—Place 25 ml of sample in 100 ml volumetric flask, dilute to mark, and mix thoroly. Pipet 10 ml aliquot into suitable flask. Add 25 ml of  $\text{HNO}_3$ , and boil on hot plate until volume is reduced to 2–3 ml; add 10 ml of  $\text{HNO}_3$ , and boil until volume is again reduced to 2–3 ml. Cool, and add 20 ml of  $\text{H}_2\text{O}$ . Add  $\text{NH}_4\text{OH}$  in slight excess, and barely dissolve precipitate formed with a few drops of  $\text{HNO}_3$ , stirring vigorously. To the hot soln add 70 ml of molybdate soln, 2. 7(a), for each 0.1 g of  $\text{P}_2\text{O}_5$  present. Digest at ca  $65^\circ$  for 1 hour, and test for complete precipitation by addition of more reagent to clear supernatant liquid. Filter, and wash with  $\text{NH}_4\text{NO}_3$  soln, 2. 7(b).

Dissolve precipitate on filter with  $\text{NH}_4\text{OH}$  (1+1) and hot  $\text{H}_2\text{O}$  and wash into a beaker to volume of not more than 100 ml. Nearly neutralize with  $\text{HCl}$ , using litmus paper as indicator. Cool, and from a buret add slowly (ca 1 drop/second, stirring vigorously) 15 ml of magnesia mixture, 2. 7(c), for each 0.1 g of  $\text{P}_2\text{O}_5$  present. After 15 min. add 12 ml of  $\text{NH}_4\text{OH}$  and allow to stand overnight. Filter, and wash precipitate with dilute  $\text{NH}_4\text{OH}$ , 2. 7(d), until washings are practically free from chlorides. Dry, burn first at low heat, and then ignite to constant weight, preferably in electric furnace, at  $950$ – $1000^\circ$ . Cool, and weigh as  $\text{Mg}_2\text{P}_2\text{O}_7$ .  $\text{Mg}_2\text{P}_2\text{O}_7 \times 0.6379 = \text{P}_2\text{O}_5$ .

(b) *Calcium*.—Using prepared soln (a), pipet a 20 ml aliquot into 400 ml beaker and dilute to 100 ml. Add 2 ml of  $\text{HCl}$ , 15 ml of 10%  $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$  soln, and a slight excess of saturated  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  soln. Heat to boiling and allow precipitate to settle at temp. just below boiling. Filter hot, wash with 1%  $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$  soln, dry, moisten with  $\text{H}_2\text{SO}_4$ , ignite gently, and weigh residue as  $\text{CaSO}_4$ .  $\text{CaSO}_4 \times 0.2944 = \text{Ca}$ .

##### Method II (98)

(Not applicable in presence of other reducing agents or of phenolic compounds.)

#### 39.200

##### REAGENTS

(a) *Bromide-bromate soln*.—See 39.28(c).

(b) *Sodium thiosulfate soln*.—0.1 N. See 39.3(b).

(c) *Potassium iodide soln*.—20 g/100 ml.

#### 39.201

##### DETERMINATION

Transfer 50 ml of the sirup, measured in 50 ml volumetric flask, to 250 ml volumetric flask. Wash the 50 ml flask with several portions of  $\text{H}_2\text{O}$ , adding washings to 250 ml flask, finally making to mark with  $\text{H}_2\text{O}$  and mixing well. (This procedure is followed in the case of the sirup of  $\text{NH}_4\text{H}_2\text{PO}_4$ . For sirups containing larger quantities of hypophosphites the original 50 ml may be diluted to 500 ml in a volumetric flask.) Transfer 50 ml aliquot to 250 ml volumetric flask and make to mark with  $\text{H}_2\text{O}$ , again mixing well. Of this soln, transfer a 50 ml aliquot (equivalent to 2 ml of sirup) to glass-stoppered 250 ml flask, add 50 ml of the  $\text{KBr-KBrO}_3$  soln and 20 ml of 10%  $\text{H}_2\text{SO}_4$ ; stopper, shake well, and let stand 2 hours. Add 10 ml of the  $\text{KI}$  soln, shake flask, and titrate liberated  $\text{I}$  with the  $\text{Na}_2\text{S}_2\text{O}_3$  soln until a straw color

appears; add 2 ml of starch soln, 6.3(e), and titrate until the soln becomes colorless. Conduct a blank determination in same way. 1 ml of 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3 = 0.00165$  g of  $\text{H}_3\text{PO}_2$ ; 1 ml of 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3 = 0.00208$  g of  $\text{NH}_4\text{H}_2\text{PO}_2$ .

## 39.202

## IODINE (89)—OFFICIAL

Transfer a quantity of sample that contains not more than 0.1 g of the iodide (0.05 g is ample) to a crucible, preferably Ni, and add 2 or 3 g of solid KOH. If sample is a solid, add 10–15 ml of alcohol before adding the KOH. Dry, and char thoroly. (Use as low a temp. as possible in order to prevent loss of I, in no event more than dull redness.) Extract charred mass with hot  $\text{H}_2\text{O}$ , filter into Erlenmeyer flask, and wash well with hot  $\text{H}_2\text{O}$ .

Neutralize filtrate with  $\text{H}_2\text{SO}_4$  (1+1), make alkaline again with 4% NaOH soln, and add 1 ml in excess. Heat to boiling and add saturated  $\text{KMnO}_4$  soln slowly until  $\text{KMnO}_4$  color remains after several minutes' boiling. Then add ca 0.5 ml in excess, continue boiling ca 5 min., and allow to cool. Add a few ml of alcohol and place on steam bath. ( $\text{KMnO}_4$  color should be bleached; if it is not, add a little more alcohol.) When precipitate has settled, filter and wash with hot  $\text{H}_2\text{O}$ . After cooling, add 1–2 g of KI (crystals), acidify with HCl, and titrate with 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3$  soln. 1 ml of 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3 = 0.00277$  g of KI, 0.00250 g of NaI, or 0.00212 g of I.

## 39.203

## FREE IODINE IN IODINE OINTMENT (100)—TENTATIVE

Weigh (to 1 mg) ca 2 g of ointment, and transfer to 250 ml I flask. Melt on water bath (not above  $70^\circ$ ), add 30 ml of  $\text{CHCl}_3$ , mix well, and then add 30 ml of  $\text{H}_2\text{O}$ . (All of base should be dissolved in the  $\text{CHCl}_3$  before  $\text{H}_2\text{O}$  is added.) Titrate with 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3$ , using starch indicator, 6.3(e). Approach end point dropwise, shaking flask vigorously to make sure that all I has been extracted from  $\text{CHCl}_3$  layer. 1 ml of 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3 = 0.01269$  g of I.

## 39.204

## POTASSIUM IODIDE IN IODINE OINTMENT (101)—TENTATIVE

Pour liquids from free I determination, 39.203, into 500 ml I flask, rinsing flask with 200 ml of  $\text{H}_2\text{O}$ , divided into several portions. (It is desirable to maintain this volume within rather narrow limits.) Add 0.5 ml of 0.2% alcoholic p-ethoxychrysoidin indicator and 1–4 drops (to neutralize) of 0.1 *N* NaOH. (Aqueous layer should now be clear yellow.) Titrate with 0.1 *N*  $\text{AgNO}_3$ , approaching end point dropwise and rotating flask frequently. (The  $\text{AgNO}_3$  soln causes turbidity due to formation of colloidal AgI and development of reddish-brown color similar to that observed in over-titrated Volhard determination. End point, which is produced by 1 drop of the  $\text{AgNO}_3$  soln, is characterized by flocculation of the colloidal AgI and complete disappearance of the reddish-brown tinge, leaving an almost clear, pale yellow supernatant liquid.) 1 ml of 0.1 *N*  $\text{AgNO}_3 = 0.0166$  g of KI.

## ETHYLAMINO BENZOATE (102)—TENTATIVE

## 39.205

## REAGENTS

- (a) *Potassium bromide-bromate soln.*—0.1 *N*. See 39.28(c).
- (b) *Sodium thiosulfate soln.*—0.1 *N*. Standardize according to 39.3(b).
- (c) *Potassium iodide soln.*—See 39.28(b).
- (d) *Starch indicator.*—See 6.3(e).
- (e) *Dilute hydrochloric acid.*—(1+19).

## 39.206

## DETERMINATION

(a) *In the pure drug*.—Accurately weigh 0.12–0.15 g of sample into an I flask and dissolve with 10 ml of HCl and ca 200 ml of H<sub>2</sub>O. Add the KBr-KBrO<sub>3</sub> soln from a buret until slight excess is present as evidenced by light yellow color. Stopper flask, shake, and allow to stand 5 min. Add 5 ml of the KI soln, avoiding loss of Br, stopper flask, and shake. Titrate liberated I with the Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> soln, using starch soln as indicator. From amount of KBr-KBrO<sub>3</sub> soln used calculate percent of ethylaminobenzoate.

1 ml of 0.1 N KBr-KBrO<sub>3</sub> = 0.004127 g of ethylaminobenzoate.

(b) *In ointments*.—Accurately weigh 2.5–3 g of ointment (sufficient to provide 0.12–0.15 g of ethylaminobenzoate for titration) in small beaker, dissolve in benzene by warming on steam bath, and transfer to separator, using in all ca 50 ml of the solvent. Wash beaker with 50 ml of the HCl soln, pour into separator, and extract ethylaminobenzoate. Make 3 additional extractions with 50 ml portions of the HCl. Wash each acid extract successively in second separator by shaking with ca 20 ml of petroleum benzine and combine acid extracts in I flask. Test for complete extraction by making one additional extraction and titrating with the standard KBr-KBrO<sub>3</sub> soln. Continue as directed under (a), beginning "Add the KBr-KBrO<sub>3</sub> . . ." and calculate percent of ethylaminobenzoate.

## 39.207

## SEDORMID (109)—TENTATIVE

Ascertain average weight of number of tablets and reduce to fine powder. Transfer to small beaker a weighed portion of powder, representing ca 0.2 g of sedormid. Add warm CHCl<sub>3</sub> and filter thru rapid filter paper into weighed beaker. Repeat until all the sedormid is extracted. Evaporate CHCl<sub>3</sub> soln on steam bath with aid of electric fan, removing beaker from bath just before last portions evaporate to avoid decrepitation. Add few ml of anhydrous ether and evaporate, taking same precautions as before. Heat 10–15 min. at 100°, cool in desiccator, and weigh. Determine melting point to check purity of residue (should be 194–197°).

## IODOFORM (104)—OFFICIAL

## 39.208

## REAGENTS

(a) *Ammonium thiocyanate soln*.—0.05 N. Standardize against 0.1 N AgNO<sub>3</sub>, using an equal volume of alcohol and 3 ml of FeNH<sub>4</sub>(SO<sub>4</sub>)<sub>2</sub> soln as indicator.

(b) *Ferric ammonium sulfate indicator*.—Dissolve 8 g of FeNH<sub>4</sub>(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O in 100 ml of H<sub>2</sub>O.

## 39.209

## DETERMINATION

Weigh accurately ca 0.25 g of CHI<sub>3</sub> and transfer quantitatively to 200 ml Erlenmeyer flask. Add 40 ml of alcohol, swirl gently until the CHI<sub>3</sub> is dissolved, filter if necessary, and immediately add 40 ml of 0.1 N AgNO<sub>3</sub> and 10 ml of HNO<sub>3</sub>. Swirl gently ca 5 min., allow to stand at room temp. 2–3 hours, and then swirl occasionally as aid in flocculating the AgI. Titrate excess 0.1 N AgNO<sub>3</sub> with 0.05 N NH<sub>4</sub>CNS, using 3 ml of the FeNH<sub>4</sub>(SO<sub>4</sub>)<sub>2</sub> indicator. 1 ml of 0.1 N AgNO<sub>3</sub> = 0.01313 g of CHI<sub>3</sub>. Or, filter, collecting the AgI on dried and accurately weighed Gooch crucible, wash with H<sub>2</sub>O and finally with alcohol, and dry to constant weight at ca 125°. 1 g of AgI = 0.5590 g of CHI<sub>3</sub>.

## 39.210

## IODOFORM OINTMENT (105)—OFFICIAL

Transfer ca 2.5 g of sample to tared 50 ml beaker and weigh. Add 5 ml of  $\text{CHCl}_3$ , stir gently with glass rod, and transfer bulk of undissolved ointment and the  $\text{CHCl}_3$  soln to 250 ml flask having ground-glass stopper. Add 5 ml of  $\text{CHCl}_3$  to ointment remaining in beaker and stir until dissolved. Add soln to contents of flask and finally wash beaker three times, using not more than 5 ml of  $\text{CHCl}_3$  each time, and add washings to contents of flask. Or, weigh sample in small, tared glass capsule, drop capsule with contents into 250 ml flask having ground-glass stopper, and add not more than 20 ml of  $\text{CHCl}_3$ . (Use glass capsule only in volumetric determination.) Swirl gently until all ointment is dissolved. Add 40 ml of 0.1 *N* alcoholic  $\text{AgNO}_3$  and swirl to wash down any  $\text{CHI}_3$  that may adhere to sides of flask. Slowly add 10 ml of  $\text{HNO}_3$  and allow to stand at room temp. ca 18 hours. Titrate excess of 0.1 *N* alcoholic  $\text{AgNO}_3$  with 0.05 *N*  $\text{NH}_4\text{SCN}$ , 39.208(a), using 3 ml of  $\text{FeNH}_4(\text{SO}_4)_2$  indicator, 39.208(b), shaking mixture vigorously near end of titration. 1 ml of 0.1 *N*  $\text{AgNO}_3$  = 0.01313 g of  $\text{CHI}_3$ .

For gravimetric determination use an ordinary Erlenmeyer flask in place of the flask having ground-glass stopper. Weigh ointment base into 100 ml beaker and add  $\text{CHCl}_3$ . When ointment base has dissolved, filter thru Gooch crucible, using suction. Wash beaker and crucible once with alcohol. Wash crucible several times with  $\text{CHCl}_3$  without using suction. Collect filtrate in an Erlenmeyer flask and add 40 ml of 0.1 *N*  $\text{AgNO}_3$  and 10 ml of  $\text{HNO}_3$  in small portions. Allow mixture to stand 18 hours. Collect the  $\text{AgI}$  on weighed Gooch crucible, using suction. Wash with  $\text{H}_2\text{O}$  and then with alcohol. Finally wash repeatedly with  $\text{CHCl}_3$  without suction. Dry the Gooch crucible and contents at ca 125° to constant weight. 1 g of  $\text{AgI}$  = 0.5590 g of  $\text{CHI}_3$ .

## 39.211

## IODOFORM GAUZE (106)—OFFICIAL

Weigh in tared weighing bottle with ground-glass stopper a sample of  $\text{CHI}_3$  gauze containing ca 1 g of  $\text{CHI}_3$ . ( $\text{CHI}_3$  gauze is usually moist and loses weight rapidly when exposed to air.) Transfer to 150 ml beaker, add ca 75 ml of alcohol, and stir until  $\text{CHI}_3$  is dissolved. Filter into 200 ml volumetric flask, draining the alcoholic soln with aid of pressure upon gauze. Wash 4 or 5 times, using 25 ml of alcohol each time, filter washings, and finally make to volume with alcohol. Pipet a 40 ml aliquot into 200 ml Erlenmeyer flask and immediately add 40 ml of 0.1 *N*  $\text{AgNO}_3$  and 10 ml of  $\text{HNO}_3$ . Proceed as directed under 39.210, beginning "allow to stand at room temp."

## MERCUROUS CHLORIDE (CALOMEL) IN TABLETS (107)—OFFICIAL

## 39.212

## REAGENT

*Standard iodine soln.*—0.1 *N*. Dissolve ca 14 g of I in soln containing 18 g of KI in 100 ml of  $\text{H}_2\text{O}$ , and dilute to 1 liter. Standardize this soln against standard  $\text{Na}_2\text{S}_2\text{O}_3$  soln, 39.3(b).

## 39.213

## DETERMINATION

Count and weigh a representative number of tablets. Pulverize a quantity of tablets and weigh accurately a sufficient portion of well-mixed sample to represent 0.19–0.26 g (3–4 grains) of  $\text{HgCl}_2$ . Transfer to a 200 ml glass-stoppered Erlenmeyer flask, add ca 50 ml of  $\text{H}_2\text{O}$ , acidify with acetic acid, and after soluble fillers have dissolved decant with aid of suction thru a tightly packed asbestos mat placed on plate of a Caldwell crucible. Wash once with  $\text{H}_2\text{O}$  by decantation, then successively with alcohol and ether. Transfer removable plate holding mat and insoluble material to

original flask, washing into flask any insoluble material adhering to sides of crucible. Add 2.5 g of KI, 10 ml of  $H_2O$ , and then 30 ml of the standard I soln. Allow mixture to stand, with frequent and fairly vigorous agitation, ca 1.5 hours, or until soln of calomel is complete. Titrate with the standard thiosulfate soln, 39.3(b), and add ca 1 ml in excess. Titrate back with the standard I soln, using starch indicator, 6.3(e), until a permanent blue color is obtained. 1 ml of 0.1  $N$  I = 0.02361 g of HgCl.

**39.214 CALOMEL IN CALOMEL OINTMENTS (108)—OFFICIAL**

Weigh accurately ca 1 g of ointment, transfer to 250 ml glass-stoppered Erlenmeyer flask, and treat with ca 50 ml of  $CHCl_3$ . When base is dissolved, decant thru dry closely packed asbestos mat in Caldwell crucible, using light suction. Wash flask and contents several times with 20 or 30 ml portions of  $CHCl_3$ , decanting thru crucible. Allow any residual  $CHCl_3$  in flask to evaporate and transfer asbestos mat and contents to flask, wiping sides of crucible and mouth of flask with a damp piece of filter paper and adding it to contents of flask. Add 2.5 g of KI and 50 ml of 0.1  $N$  I soln, 39.212, stopper, and mix contents well. Allow flask to stand ca 1.5 hours or until soln of calomel is complete, agitating it frequently and fairly vigorously. Titrate with 0.1  $N$   $Na_2S_2O_3$ , adding 1 or 2 ml in excess and using starch as indicator, 6.3(e). When all traces of I have disappeared, titrate back with the standard I soln until a blue color is obtained. 1 ml of 0.1  $N$  I = 0.02361 g of HgCl.

**39.215 MERCUROUS IODIDE IN TABLETS (109)—OFFICIAL, FIRST ACTION**

Weigh accurately a sufficient portion of well-mixed powdered sample to represent 0.19–0.26 g (3–4 grains) of HgI. Transfer sample to 200 ml glass-stoppered flask, add ca 50 ml of  $H_2O$ , acidify with acetic acid, and after soluble fillers have dissolved, decant with aid of suction thru tightly packed asbestos mat placed on plate of a Caldwell crucible. Wash once with  $H_2O$  by decantation, then successively with alcohol and ether. Transfer removable plate holding mat and insoluble material to original flask, washing into flask any insoluble material adhering to sides of crucible. Add 2.5 g of KI and 30 ml of I soln, 39.212. Allow mixture to stand, with frequent and fairly vigorous agitation, ca 1.5 hours, or until soln of HgI is complete. Titrate with standard  $Na_2S_2O_3$  soln, 39.3(b), and add 1 or 2 ml in excess. When all traces of I have disappeared, titrate back with standard I, using starch indicator. 1 ml of 0.1  $N$  I = 0.03275 g of HgI.

NOTE: Some commercial tablets are difficult to filter thru the asbestos mat without loss of HgI. A few drops of alumina cream, 34.19(b), washed free from  $NH_3$ , placed on the mat before filtration is started, satisfactorily prevents loss, tho it retards filtration.

**MERCUROCHROME (110)—TENTATIVE**

**39.216 Tests for Purity**

(a) Acidify a portion of the mercurochrome soln with 10%  $H_2SO_4$  and filter off precipitate. Filtrate is colored only slightly yellow.

(b) Pass  $H_2S$  into a portion of filtrate. No precipitate or coloring occurs.

(c) Add a few ml of 10%  $HNO_3$  to another portion of filtrate and add  $AgNO_3$  soln. No precipitate forms.

**39.217 Total Solids in Solution (110)—Tentative**

Pipet 10 ml of the mercurochrome soln into tared, extra-wide-form weighing bottle and evaporate to dryness on steam bath. Allow to dry overnight in the open bottle in desiccator containing  $H_2SO_4$ . Weigh.



## 39.218

*Determination of Mercury (110)—Tentative*

Pipet 10 ml of ca 2% soln of the mercurochrome into 500 ml tall-form beaker and evaporate to dryness on steam bath (or weigh accurately ca 0.2 g of the powder). Dissolve residue in 4 ml of  $H_2O$  and add slowly, with constant mixing, 10 ml of  $H_2SO_4$ . Incline beaker and add cautiously small portions of  $KMnO_4$  (finely pulverized), mixing after each addition, until considerable excess has been added, as indicated by deep purple color of mixture. Allow to stand 30 min., occasionally mixing, at end of which time mixture should still retain its purple color. Add 100 ml of  $H_2O$  and mix thoroly. Add small portions of oxalic acid (finely pulverized), mixing after each addition, until the soln is clear. Filter thru small filter into 400 ml beaker, wash original beaker and filter until filtrate measures ca 200 ml, and pass  $H_2S$  thru soln 20 min. Warm on steam bath until precipitate of  $HgS$  settles quickly after stirring, and again pass  $H_2S$  thru warm soln 5 min. Filter soln immediately into weighed Gooch crucible, and wash precipitate on filter well with  $H_2O$ , three times with the alcohol, and then with 4 or 5 portions of  $CCl_4$  or  $CS_2$ , allowing liquid to run thru crucible without suction; finally wash with ether. Dry precipitate to constant weight at  $100^\circ$ , and weigh as  $HgS$ . Test dried precipitate qualitatively for  $Hg$  and other heavy metals. If any difficulty is experienced by slow filtration during the washing with  $H_2O$ , allow precipitate to drain and wash once with alcohol, then continue as directed.  $HgS \times 0.8622 = Hg$ .

## 39.219 MERCURY IN OINTMENT OF MERCURIC NITRATE (111)—OFFICIAL, FIRST ACTION

Transfer to 200–300 ml Erlenmeyer flask 3–5 g of sample accurately weighed, using glass or bone spatula. Add 40 ml of  $HNO_3$  (1+1) and a few glass beads and insert a short-stemmed funnel into neck of flask. Boil gently 1–1.5 hours on hot plate or over low flame. With latter use piece of asbestos having a circular hole under an asbestos wire gauze. Add 30 ml of  $H_2O$ , using part to wash funnel. Cool sufficiently to cause unconsumed fat to form a hard cake (ca  $20^\circ$  or below). Filter thru 11 cm filter into 200 ml volumetric flask. Wash fat, flask, and filter, using ca 100 ml of 1%  $HNO_3$ . Make to volume and mix well. Reserve fat to test for complete extraction as directed below.

Test for complete extraction of the  $Hg$  from the fat and its removal from the filter, etc., by repeating the  $HNO_3$  (1+1) digestion ca 30 min. on residual fat in flask or on filter, completing this as a separate determination, including the  $KMnO_4$  digestion. Add any titration in excess of 1 to 2 drops (ca 0.05–0.08 ml of 0.1  $N$   $NH_4CNS$ ) resulting from this test portion to that obtained by titrating the main extract.

Transfer 100 ml aliquot to 500 ml Erlenmeyer flask. Add 7 ml of  $HNO_3$ , 5 ml of  $H_2SO_4$ , and 2 g of powdered  $KMnO_4$ , and rotate to dissolve. Heat just to boiling over low flame or on hot plate. Boil gently 45 min., maintaining excess of  $KMnO_4$ , indicated by dark purple color. (An excess is essential.) When adding  $KMnO_4$  to boiling liquid use smaller portions (ca 0.5 g or less) to avoid loss due to frothing.

**CAUTION:** Use of greater excess of  $KMnO_4$  than is necessary is not objectionable, but it will require proportionately more  $H_2O_2$  to remove it and the  $MnO_2$  at end of digestion. Usually ca 10 g is required. Rate of consumption and total  $KMnO_4$  consumed seem to vary with temp., organic matter present, and period of heating. The large amount of  $MnO_2$  formed may lead to wrong conclusion concerning the color indicative of an excess of  $KMnO_4$ . Frequent examination of the soln is necessary. The observation of this color is aided when the analyst looks thru the supernatant liquid toward a white background while holding the container in an inclined position.

Remove excess  $KMnO_4$  and dissolve  $MnO_2$  by adding  $H_2O_2$  (5–10% prepared

from 30%) dropwise to the hot soln. When colorless add 2%  $\text{KMnO}_4$  soln slowly until a faint pink or brown persists ca 1 min. If a large amount of  $\text{MnO}_2$  forms at this point, again use  $\text{H}_2\text{O}_2$  sparingly, then  $\text{KMnO}_4$  to discharge the  $\text{H}_2\text{O}_2$ . Discharge the color from the last  $\text{KMnO}_4$ , including a weak brown color from  $\text{MnO}_2$ , by adding dropwise just sufficient 8%  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ . Cool to ca  $20^\circ$ , add 3 ml of 0.5 *N*  $\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$  and titrate with the standard  $\text{NH}_4\text{CNS}$ . 1 ml of 0.1 *N*  $\text{NH}_4\text{CNS}$  = 0.01003 g of Hg.

### 39.220 MERCURY IN MERCURIAL OINTMENT (118)—OFFICIAL, FIRST ACTION

After mixing the ointment thoroly with a glass rod, avoiding contact with metals, weigh 1 g of material into Erlenmeyer flask. Add 20 ml of  $\text{H}_2\text{O}$  and 20 ml of  $\text{HNO}_3$ , and heat gently over small flame until red fumes cease to evolve. Cool, and decant aqueous soln from the ointment base into separator. Wash ointment base with 50 ml of boiling  $\text{H}_2\text{O}$ , cool, and decant into separator. Repeat washing until all Hg is removed. Shake combined solns in separator with 50 ml of ether. Transfer aqueous soln to an Erlenmeyer flask. Wash the ether three times with 10 ml portions of  $\text{H}_2\text{O}$  until the Hg is removed, adding washings to flask. Add 3 ml of  $\text{FeNH}_4(\text{SO}_4)_2$  soln, 39.208(b), and titrate with 0.1 *N*  $\text{NH}_4\text{CNS}$ . 1 ml of 0.1 *N*  $\text{NH}_4\text{CNS}$  = 0.01003 g of Hg.

### NITRITES IN TABLETS (119)—OFFICIAL, FIRST ACTION

#### 39.221 PREPARATION OF SAMPLE—See 39.1

#### 39.222 DETERMINATION

Transfer to 100 ml volumetric flask a quantity of powdered sample equivalent to ca 1 g of  $\text{NaNO}_2$ , add  $\text{H}_2\text{O}$  to mark, and mix thoroly. Filter thru dry filter, rejecting first 10 ml. Transfer 50 ml aliquot to 200 ml volumetric flask. Add in order 10 ml of saturated  $\text{KClO}_3$  soln, and slowly, with shaking, 10 ml of  $\text{HNO}_3$  soln (1+1), and allow to stand 30 min. Add 50 ml of 0.1 *N*  $\text{AgNO}_3$  and make to mark with  $\text{H}_2\text{O}$ . After mixing thoroly, filter thru a dry filter, rejecting first 20 ml of filtrate. To 100 ml of filtrate add 2 ml of  $\text{FeNH}_4(\text{SO}_4)_2$  soln, 39.208(b), and titrate with 0.05 *N*  $\text{NH}_4\text{CNS}$ , 39.208(a). Make a blank determination, and correct if necessary. 1 ml of 0.1 *N*  $\text{AgNO}_3$  = 0.0207 g of  $\text{NaNO}_2$ .

NOTE: Correct for any Cl that may be present in sample. If a large quantity of insoluble excipient is present, pipet 100 ml of  $\text{H}_2\text{O}$  into a flask with the powdered sample in order to avoid any error in volume.

### 39.223 PHENOLSULFONATES (114)—OFFICIAL

Dissolve sample (equivalent to ca 0.8 g of phenolsulfonate) in ca 30 ml of  $\text{H}_2\text{O}$  and add 5 ml of  $\text{HCl}$ . Titrate with 0.4 *N*  $\text{KBr-KBrO}_3$  soln (11.134 g of  $\text{KBrO}_3$  + 50 g of  $\text{KBr}$  diluted to 1 liter with  $\text{H}_2\text{O}$ , standardized against 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_5$ ). The Br will be absorbed very rapidly at first, but as titration proceeds, absorption becomes slower and slower. Titrate as far as possible with no other indicator than fading of Br yellow. (Usually this will be within 1–4 ml of end point.) Then use methyl orange (0.1%), dropwise, adding no new indicator until previous drop has practically faded. After adding the reagent, wait a sufficient time for absorption of the Br before adding more methyl orange (10 seconds at first, 15 seconds at end of titration), because in the presence of dibromophenolsulfonic acid the action of Br on methyl orange is much slower than normal. End point is reached when, after waiting 15 seconds for absorption of last drop of Br and adding a drop of methyl orange, the latter fades very appreciably in 10 seconds. It is always best, after the methyl orange has faded, to add another drop to be sure that first drop was not added too soon. 1 ml of 0.4 *N*  $\text{KBr-KBrO}_3$  = 0.0232 g of Na phenolsulfonate.

**EFFERVESCENT POTASSIUM BROMIDE WITH CAFFEINE (115)—OFFICIAL****39.224****PREPARATION OF SAMPLE**

Powder sample, transfer immediately to dry bottle, and seal tightly. Thoroughly mix powder in bottle by rotating and shaking before removal of sample for analysis. Weigh out all needed portions as nearly at same time as possible. Avoid extreme temperatures and humidities when opening and storing samples.

**39.225****DETERMINATIONS**

(a) *Potassium bromide*.—Weigh 2.5–3 g of sample and transfer to 250 ml Erlenmeyer flask. Add 50 ml of  $H_2O$  and swirl gently, avoiding loss of soln by spattering. Acidify soln with  $HNO_3$  and then add 5 ml in excess. Add 30 ml of 0.1  $N$   $AgNO_3$ , 6.91, and 2 ml of indicator, 39.208(b). Allow mixture to stand several minutes and swirl occasionally as an aid in flocculating the  $AgBr$ . Titrate excess of 0.1  $N$   $AgNO_3$  with  $NH_4CNS$  soln, 39.208(a). 1 ml of 0.1  $N$   $AgNO_3$  = 0.0119 g of  $KBr$ .

(b) *Caffeine*.—Weigh 12–15 g of sample, transfer to separator, and slowly add 50 ml of  $H_2O$ , avoiding loss of soln by spattering. If soln is not alkaline to litmus, make basic with 5%  $NaOH$  soln. Add 50 ml of  $CHCl_3$ , shake vigorously, and filter into beaker. Repeat extraction twice, using 50 ml portions of the  $CHCl_3$  each time. Wash filter and funnel with a few ml of  $CHCl_3$  to remove any adhering caffeine. Evaporate combined  $CHCl_3$  filtrates on water bath to ca 10 ml, finally transferring residual liquid to small weighed beaker. Allow soln to evaporate by gentle heat and an air blast. Dry residue to constant weight at  $80^\circ$ , and weigh.

**SILVER PROTEINATES (116)****39.226***Acidity or Alkalinity—Tentative*

Dialyze 1 g of sample as directed under 39.228 and titrate a portion of the clear soln representing 0.5 g of the sample with either 0.02  $N$   $HCl$  or 0.02  $N$   $NaOH$ , as required, using phenolphthalein indicator. Calculate acidity as percentage of  $HCl$  and alkalinity as percentage of  $NaOH$ .

**39.227***Total Silver (117)—Official*

Place 1 g of sample, accurately weighed, in 500 ml Kjeldahl flask; add 15 ml of  $H_2SO_4$  and then 10 ml of  $HNO_3$ ; place on steam bath for a few minutes, with occasional rotation, to insure a homogeneous mixture; and boil to white fumes. Add more  $HNO_3$ , boil again to clear colorless soln, and cool. Add 100 ml of  $H_2O$  and boil until free of  $N$  oxides. Cool, dilute to 300 ml, add 5 ml of  $HNO_3$  and 5 ml of  $FeNH_4(SO_4)_2$  soln, 39.208(b), and titrate with 0.1  $N$   $NH_4CNS$ . 1 ml of 0.1  $N$   $NH_4CNS$  = 0.01079 g of  $Ag$ .

**39.228***Ionizable Silver Compounds (117)—Official*

Weigh a strip of commercial dialyzing tubing 55 mm wide and ca 1 foot long, wet with  $H_2O$  until uniformly pliable, shake free of adhering  $H_2O$ , and partially dry by rolling in clean paper towel. Reweigh while still moist and place in 250 ml beaker. (Sheets of dialyzing parchment paper may be used in place of tubing. Over one end of a glass tube 10 cm long and ca 2.5 cm in diam., fold and secure by means of rubber band a square piece of parchment paper in form of a sack of sufficient size to hold sample soln. Dialyzing material should be kept in humid container to prevent breaking when handled.) Weigh 1 g of sample, dissolve in 15 ml of  $H_2O$ , and transfer to dialyzing tube. Calculate, and add sufficient  $H_2O$  to beaker to make 100 ml (this

insures 20 ml in the dialyzing tube and 80 ml in the beaker). Adjust tubing to form a "U" in beaker, cover with watch-glass, and place in cool dark closet for 24 hours.

(a) *Qualitative Test*.—Test a few ml of clear, colorless soln from the beaker for Ag ions by addition of a few drops of 10% HCl and a trace of HNO<sub>3</sub>.

(b) *Quantitative Method*.—If Ag ions are present, remove 50 ml of the clear, colorless soln from beaker (representing 0.5 g of sample), dilute to 100 ml, and add 2 ml of FeNH<sub>4</sub>(SO<sub>4</sub>)<sub>2</sub> soln, 39.208(b), and the same quantity of colorless HNO<sub>3</sub>. Titrate with 0.01 N NH<sub>4</sub>CNS and calculate to percentage by weight of ionizable Ag. 1 ml of 0.01 N NH<sub>4</sub>CNS = 0.001079 g of Ag.

### 39.229 COD LIVER OIL IN EMULSIONS (118)—OFFICIAL, FIRST ACTION

Weigh into tared beaker of ca 150 ml capacity sufficient well-mixed sample to contain ca 2 g of cod liver oil. Add ca 10 g of finely powdered CaCO<sub>3</sub> and thoroly mix with stirring rod. Add 30 ml of CHCl<sub>3</sub>, thoroly mix, and decant thru dry filter into 100 ml air-dried, tared beaker. Continue to extract and wash repeatedly with 5–10 ml portions of CHCl<sub>3</sub> until filtrate is ca 60 ml. Evaporate the CHCl<sub>3</sub> on steam bath with current of air to ca 5 ml.

Continue extraction and carefully wash filter paper and funnel, filtering into 250 ml beaker until filtrate is ca 150 ml. Evaporate to ca 10 ml and transfer to first tared beaker. Repeat procedure until extraction is complete or until 25 ml of solvent upon evaporation in second tared beaker yields 0.001 g or less of residue. Evaporate CHCl<sub>3</sub> in first tared beaker and allow to remain on steam bath ca 10 min. after odor of CHCl<sub>3</sub> has disappeared. Dry in oven at not over 100° for 5 min. intervals until weight is constant or loss is 0.001 g or less.

CAUTION: Avoid prolonged heating or long exposure to air at room temp. The oil absorbs O, weight increases appreciably, and the physical constants change.

### OIL OF CHENOPODIUM (119)—OFFICIAL

#### 39.230

#### REAGENTS

(a) *Standard ferric ammonium sulfate soln*.—Dissolve 39.214 g of pure, crystallized Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O in 200 ml of H<sub>2</sub>O in liter flask, add 30 ml of H<sub>2</sub>SO<sub>4</sub>, and mix well. Weigh exactly 3.16 g of KMnO<sub>4</sub>, dissolve in 200 ml of warm H<sub>2</sub>O, and slowly add to soln in the flask, with stirring. (KMnO<sub>4</sub> soln should be just sufficient to oxidize the ferrous salt, but it is well to add the last few ml in small portions.) Cool soln and dilute to 1 liter with H<sub>2</sub>O.

(b) *Standard titanium trichloride soln*.—Add 100 ml of commercial 15–20% TiCl<sub>3</sub> soln to 200 ml of HCl, boil 1 min., cool, and dilute to 4500 ml with H<sub>2</sub>O. Place soln in container with H atmosphere provision and allow to stand 2 days for absorption of residual O. Preserve the TiCl<sub>3</sub> soln in an atmosphere of H (Chap. 21, Fig. 27), taking care to have all joints air-tight, and covering stoppers (preferably countersunk) with suitable wax. Standardize by titrating 20 ml of the FeNH<sub>4</sub>(SO<sub>4</sub>)<sub>2</sub> soln against the TiCl<sub>3</sub> soln in a protective stream of CO<sub>2</sub>, using 1 ml of 5% NH<sub>4</sub>CNS soln as indicator. 1 ml of 0.1 N FeNH<sub>4</sub>(SO<sub>4</sub>)<sub>2</sub> = 0.01545 g of TiCl<sub>3</sub>.

#### 39.231

#### DETERMINATION

Weigh 1 ml of the oil in 100 ml volumetric flask and dilute to volume with alcohol. Place 50 ml of the TiCl<sub>3</sub> soln in Erlenmeyer flask thru which current of CO<sub>2</sub> is passing. Fit flask with Bunsen valve, add 10 ml of diluted soln of the oil, close flask (with the Bunsen valve), and heat contents almost to boiling for 2 min. (Prolonged heating has no effect if contents are not boiled vigorously.) If pale violet color of the TiCl<sub>3</sub>

disappears, add more reagent to insure excess. (Formation of a white precipitate does not interfere with determination.) Add 1 ml of 5%  $\text{NH}_4\text{CNS}$  soln and titrate back excess of  $\text{TiCl}_3$  with the  $\text{FeNH}_4(\text{SO}_4)_2$  soln in  $\text{CO}_2$  atmosphere until faint, permanent, brownish red color is obtained.

Subtract quantity of  $\text{FeNH}_4(\text{SO}_4)_2$  soln used, expressed in equivalent mg of  $\text{TiCl}_3$ , from number of mg of  $\text{TiCl}_3$  taken. Difference is number of mg of  $\text{TiCl}_3$  oxidized by oil taken. Convert mg of  $\text{TiCl}_3$  oxidized into ascaridole by dividing by factor 1.284 (1 g of ascaridole is reduced by 1.284 g of  $\text{TiCl}_3$ ).

EXAMPLE: 0.9600 g of oil was made up to 100 ml and 10 ml aliquot was heated with 50 ml of the  $\text{TiCl}_3$  soln (1 ml containing 0.0034 g of  $\text{TiCl}_3$ ). It then required 5.9 ml of the reagent, each ml equivalent to 0.01545 g to  $\text{TiCl}_3$ , to back titrate. Grams of  $\text{TiCl}_3$  oxidized is numerically equal to  $(50 \times 0.0034) - (5.9 \times 0.01545)$ , or 0.07885. Weight of oil in the aliquot was 0.0960 g. Hence percentage of ascaridole

$$= \frac{0.07885 \times 100}{0.096 \times 1.284} = 72.1\%$$

## BIOASSAY OF DRUGS

### MYDRIATICS AND MYOTICS

#### *Cat-Eye Method (120)—Official*

#### 39.232

##### APPARATUS

(a) *Mohr pipets*.—1 ml, graduated in 0.1 ml, with slender tips that deliver exactly 0.05 ml per drop.

(b) *N-filled electric lamps*.—100-watt or equally intense illumination.

#### 39.233

##### ANIMALS

*Adult cats*.—In good physical condition, weighing over 1500 g, and accustomed to being handled.

#### 39.234

##### PREPARATION OF SAMPLE

Dissolve, in ca neutral  $\text{H}_2\text{O}$ , a representative number of tablets, or sufficient quantity of powder, to make soln containing 1 mg of the alkaloid per ml of soln. If the alkaloids themselves are taken, add the equivalent quantities of acid to convert them into the corresponding salts. Add 2 drops of ca 0.02 *N* acid/50 ml. of soln.

For great accuracy, results of chemical assay upon the sample should be followed in preparation of solns; when such accuracy is unnecessary, declaration of concentration on label may be accepted as basis for preparation of soln.

One drop of the respective concentrations of the following drugs is minimum effective dose:

MYDRIATICS		mg. per liter
Atropine.....		12
Hyoscyamine.....		4
Scopolamine.....		0.4
Homatropine.....		200
Cocaine.....		60
Euphthalmin.....		50,000
Ephedrine (alkaloid).....		2,500
Ephedrine salt (or synthetic ephedrine).....		50,000
Pseudoephedrine (alkaloid).....		2,500
Pseudoephedrine (salt).....		80,000
MYOTICS		
Pilocarpine.....		25,000
Physostigmine (eserine).....		10
Arecoline.....		10,000

## 39.235

## DETERMINATION OF CAT'S THRESHOLD

Place a cat ca 1 foot from 100-watt electric lamp, and determine maximum contractility of its pupils under this condition. Drop 0.05 ml of the freshly prepared standard mydriatic soln, obtained by diluting the 1 mg-per-ml soln, into outer margin of one eye, leaving other eye untreated as control. Compress inner canthus, while opening and closing lids, until fluid has apparently disappeared (10-30 seconds). Return cat to cage.

One and two hours after application (for atropine, 3 and 4 hours also), place cat under same conditions, and note any differences in diameter between pupils of treated and untreated eyes. (Satisfactory reaction is produced when pupil of treated eye is just perceptibly wider (0.5-1.0 mm) than pupil of untreated eye.) Do not use same eye for another assay for at least 24 hours.

If concentrations given fail to produce a satisfactory reaction, repeat test with more or less concentrated soln until minimum effective concentration is found. (This concentration may vary somewhat for different cats, but is essentially constant for same cat.)

## 39.236

## BIOASSAY OF UNKNOWN SOLUTIONS

Dilute the 1 mg-per-ml soln to be tested to the minimum effective concentration for the cats to be used, and drop 0.05 ml of this dilution into one eye of the cat, following same procedure as in determination of minimum effective concentration. Also prepare more or less concentrated solns and apply to one eye of each of other cats used. Test various concentrations until one is obtained that produces satisfactory mydriasis of same degree as standard soln when tested on two or more cats.

To obtain mg of alkaloid present in each ml of original soln, multiply mg/ml found to be the cat's minimum effective concentration by the dilution used. Knowing that original soln was made to contain 0.001 g of alkaloid/ml, calculate quantity of mydriatic present, and express as percentage of total alkaloid.

## ASSAY OF ERGOT (121)—TENTATIVE

(Applicable to alkaloids of ergotoxine-ergotamine group)

## 39.237

## REAGENTS

(a) *Menstruum I.*—Mix 20 ml of HCl with 490 ml of alcohol and 490 ml of H<sub>2</sub>O.

(b) *Menstruum II.*—Diluted alcohol (1+1).

(c) *Locke-Ringer soln modified.*—Dissolve 9 g of NaCl, 0.42 g of KCl, 0.24 g of CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.5 g of NaHCO<sub>3</sub>, and 0.5 g of dextrose in H<sub>2</sub>O (recently distilled from hard glass flask) and dilute to 1 liter.

## 39.238

## APPARATUS

Use an isolated organ bath similar to that described in U.S.P. XII under *Injectio Pituitarii Posterioris*, modifying it by having two glass chambers for the isolated tissues instead of one. Fix two levers, one above each chamber, to write on the recording drum. Magnification of the two levers should be approximately equal.

## 39.239

## PREPARATION OF SAMPLE

Pack the ergot, recently ground to No. 20 powder, in cylindrical percolator, and slowly percolate with purified petroleum benzine until a few drops of the percolate leave no greasy stain when evaporated from filter paper. Reject the soln, remove drug from percolator, and dry it by exposure to air.

Moisten defatted drug with 5%  $\text{NaHCO}_3$  soln and allow to stand in cold room 2–4 hours. Pack in percolator, add more  $\text{NaHCO}_3$  soln, and allow to macerate 16–24 hours in cold room. Percolate slowly with  $\text{H}_2\text{O}$  until percolate is found by physiological tests to be practically free from amines. Allow to drain completely, remove all but lowest inch of marc from percolator, and remove  $\text{H}_2\text{O}$  from it by strong expression or by centrifuging. Moisten drug with small quantity of Menstruum I. Repack drug in percolator and macerate overnight in cold room. Add remainder of Menstruum I, and when this has just disappeared from surface gradually add Menstruum II, constantly maintaining stratum of liquid above drug. When liquid begins to drop from percolator, close lower orifice, and having closely covered percolator, macerate 48 hours in cold room, and then allow percolation to proceed slowly, gradually adding Menstruum II until drug is exhausted. Reserve first 850 ml of percolate, recover alcohol from remainder of percolate, and concentrate residue to soft extract at temp. not exceeding  $60^\circ$  (preferably in vacuum distillation apparatus). Dissolve extract in reserved portion, mix thoroly, and assay a portion by method given below. From results thus obtained adjust volume of finished fluidextract by addition of Menstruum II to make it conform to required biological standard.

## 39.240

## DETERMINATION

Use nonpregnant female rabbits weighing 2 kg or more, and which are at least 3 weeks past parturition. Kill animal by blow on head, cut throat, and suspend by hind legs until hemorrhage ceases. Remove uterus. Cut a piece ca 1 cm in length from one uterine horn. (Remainder of uterine horns may be placed between pieces of cotton wool dampened with the modified Locke-Ringer soln and kept, properly covered, at temp. of  $40\text{--}50^\circ\text{F}$  for any subsequent tests within next 2 days.) Place the piece of uterine horn on convex surface of watch-glass and cut it open along the line of mesometric attachment. Unfurl, and cut away the sides so that a piece 8–10 mm wide farthest from the mesometrium remains. Divide this piece longitudinally into two equal parts and suspend each in one of the glass containers of the bath holding the Locke-Ringer soln. Weight recording levers so as to induce relaxation of the muscle. To each bath add 0.02 mg of epinephrine (as the hydrochloride in soln). If necessary, increase simultaneously the dose in each bath by increments of 0.01 or 0.02 mg until a contraction that is maintained 2–3 min. is produced. Adjust weights on levers so that extent of contraction in each bath is similar and conspicuously greater than any spontaneous contractions. Spontaneous contractions increase in amplitude as muscle remains in bath so that weighting should as a rule be greater than at first seems necessary. At a noted time add to one bath a dose of a specific alkaloid. (This may be 0.4 ml of a soln of ergotamine tartrate or ergotoxine phosphate, 1 in 30,000. Concentration may vary from 1 part in 10,000 to 1 part in 200,000, depending upon response obtained in muscle.) 30 seconds later add to other bath 0.4 ml of the unknown soln diluted approximately 30 times; 10 min. from the time of the additions to each bath add epinephrine to each bath in dosage found to give a previously satisfactory contraction. Note contraction produced on each muscle. If contraction has been reduced to same extent in both baths the concentration of the specific alkaloid in each bath is the same. If the contractions are not the same, repeat procedure, using fresh uterine strips, the same dose of epinephrine and larger or smaller doses of the ergot preparation as indicated by previous test.

## MICROCHEMICAL TESTS FOR ALKALOIDS—OFFICIAL

## 39.241

## REAGENTS

(a) *Ammoniacal silver nitrate soln.*—Dissolve 2 g of  $\text{AgNO}_3$  in 100 ml of 5%  $\text{NH}_4\text{OH}$ . Prepare fresh soln each time used.

- (b) *Ammonium hydroxide soln.*—10%  $\text{NH}_3$ .
- (c) *Ammonium thiocyanate soln.*—Dissolve 5 g of  $\text{NH}_4\text{CNS}$  in 100 ml of  $\text{H}_2\text{O}$ .
- (d) *Disodium phosphate soln.*—Dissolve 5 g of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  in 100 ml of  $\text{H}_2\text{O}$ .
- (e) *Gold bromide soln.*—Dissolve 1 g of  $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$  and 1.5 ml of 40%  $\text{HBr}$  in 18 ml of  $\text{HCl}$ . (Saturated aqueous  $\text{NaBr}$  soln may be substituted for the  $\text{HBr}$ .)
- (f) *Gold chloride soln.*—Dissolve 1 g of  $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$  in 20 ml of  $\text{H}_2\text{O}$ .
- (g) *Hydrochloric acid.*—5%.
- (h) *Kraut soln.*—Dissolve 8 g of  $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$  in 20 ml of  $\text{HNO}_3$ , sp. g. 1.18. Dissolve 27.2 g of  $\text{KI}$  in 50 ml of  $\text{H}_2\text{O}$ . Mix the solns and dilute to 100 ml.
- (i) *Lead iodide soln.*—To 1:3  $\text{K}$  acetate soln in  $\text{H}_2\text{O}$ , add 1 drop of methyl red indicator and acetic acid until yellow color changes to orange; then, while gently warming, saturate with  $\text{PbI}_2$ , cool, and filter.
- (j) *Marmé soln.*—Dissolve 3 g of  $\text{CdI}_2$  in 18 ml of  $\text{H}_2\text{O}$  containing 6 g of  $\text{KI}$ .
- (k) *Mercuric chloride soln.*—Dissolve 5 g of  $\text{HgCl}_2$  in 100 ml of  $\text{H}_2\text{O}$ .
- (l) *Mercuric chloride-sodium chloride soln.*—Dissolve 5 g of  $\text{HgCl}_2$  and 0.75 g of  $\text{NaCl}$  in 100 ml of  $\text{H}_2\text{O}$ .
- (m) *Platinic chloride soln.*—Dissolve 5 g of  $\text{H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$  in 100 ml of  $\text{H}_2\text{O}$ .
- (n) *Potassium ferrocyanide soln.*—Dissolve 5 g of  $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$  in 100 ml of  $\text{H}_2\text{O}$ .
- (o) *Potassium hydroxide soln.*—Dissolve 5 g of  $\text{KOH}$  in 100 ml of  $\text{H}_2\text{O}$ .
- (p) *Potassium iodide soln.*—Dissolve 5 g of  $\text{KI}$  in 100 ml of  $\text{H}_2\text{O}$ .
- (q) *Potassium permanganate soln.*—Dissolve 1 g of  $\text{KMnO}_4$  in 100 ml of  $\text{H}_2\text{O}$ .
- (r) *Reinecke salt soln.*—Dissolve 0.1 g of  $\text{NH}_4[\text{Cr}(\text{NH}_3)_2(\text{SCN})_4] \cdot \text{H}_2\text{O}$  and 0.03 g of hydroxylamine hydrochloride in 10 ml of alcohol. Filter and store in refrigerator. (Reagent is stable for 6 months or more.)
- (s) *Sodium benzoate soln.*—Dissolve 5 g of  $\text{Na}$  benzoate in 100 ml of  $\text{H}_2\text{O}$ .
- (t) *Sodium carbonate soln.*—Dissolve 5 g of  $\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$  in 100 ml of  $\text{H}_2\text{O}$ .
- (u) *Sodium iodide soln.*—Dissolve 5 g of  $\text{NaI}$  in 100 ml of  $\text{H}_2\text{O}$ .
- (v) *Sodium nitroprusside.*— $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$  crystals.
- (w) *Wagner (iodine) soln.*—Dissolve 1.27 g of  $\text{I}$  and 2 g of  $\text{KI}$  in 5 ml of  $\text{H}_2\text{O}$  and dilute to 100 ml.
- (x) *Zinc chloride soln.*—Dissolve 5 g of  $\text{ZnCl}_2$  in 100 ml of  $\text{H}_2\text{O}$ .
- (y) *Zinc potassium iodide soln.*—Dissolve 5 g of  $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$  and 20 g of  $\text{KI}$  in 100 ml of  $\text{H}_2\text{O}$ .

## 39.242

## PREPARATION OF SAMPLES

- (a) *Controls.*—Dissolve 1 mg of the pure alkaloidal salt in 2 drops of  $\text{H}_2\text{O}$  to make ca 1–100 soln.
- (b) *Alkaloids in compounds.*—Separate the alkaloid in pure form by extracting it from ammoniacal soln with suitable immiscible solvent, and evaporate solvent. To 1 mg of residue add, dropwise, 0.1  $N$   $\text{HCl}$ , avoiding excess of acid, and dilute with  $\text{H}_2\text{O}$ , if necessary, to ca same alkaloidal concentration as is specified in (a).
- (c) *Hypodermic tablets.*—Dissolve a portion of a tablet in  $\text{H}_2\text{O}$  and dilute with  $\text{H}_2\text{O}$  to ca same alkaloidal concentration as specified in (a).

## 39.243

## IDENTIFICATION

Place a drop of the alkaloidal soln on clean glass slide, add a drop of reagent by means of clean glass rod, and without stirring or covering examine under microscope, using low power. Magnification of 100–150 is suitable. Note kind of crystals formed and compare their characteristics with descriptions given and also with a control, 39.244.



## 39.244

*Characteristics of Microchemical Tests for Alkaloids*

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Aconitine (122)	Sodium carbonate	In 1:3000 soln heated to 50° in test tube. Small transparent hexagonal plates, also rods in contact.
Apomorphine (123)	Potassium iodide Gold chloride Hydrochloric acid	1:50. Small crystals, which have sharp clear cut angles like those of a diamond. Red-brown, fine needles, in dense masses in all solns to 1:10,000. 1:50. Small rods singly and in clusters.
Arecoline (124)	Kraut	Red, rhombic crystals.
Atropine (125)	Wagner	Small dark rods and triangular plates form in great numbers, singly and in groups.
Benzedrine (126)	Gold chloride	1:100 yellow square plates of various sizes.
Benzocaine (127) (Ethyl amino-benzoate)	Potassium ferro-cyanide	1:100 in dilute HCl. Colorless, irregular plates and rods.
Benzyl morphine (128) (Peronine)	Potassium iodide  Ammonium thiocyanate Hydrochloric acid	1:200. Dense rosettes of needles. Crystals are formed readily in dilute solns (1:1000) in form of sheaves of needles. 1:200. Rosettes and sheaves of needles in acid or neutral soln. 1:100. Rods, usually notched at ends and often in rosettes, are formed on stirring.
Berberine (129)	Hydrochloric acid	Saturated soln; fine yellow needles. (Avoid excess reagent.)
Brucine (130)	Potassium iodide Mercuric chloride	Long masses of transparent, rectangular plates; also rosettes of thin plates. Small, dense rosettes.
Caffeine (130)	Mercuric chloride	Clusters of long, radiating, needle-shaped crystals.
Choline (131)	Reinecke salt  Platinic chloride and sodium iodide	Add 1 drop acetone to 1 drop H <sub>2</sub> O soln of base. Stir, add 1 drop reagent and stir again. 1:100. Thin, hexagonal plates and star-shaped forms. 1:1000-1:10,000. Six-sided, more coffin-shaped plates; sometimes rosette aggregates of plates on edge, resembling needles. 1:100 in H <sub>2</sub> O. Add 1 drop H <sub>2</sub> PtCl <sub>6</sub> soln, stir, add small drop NaI soln without stirring. Small black rectangular prisms and slender black rods.
Cinchonidine (132)	Sodium benzoate Platinic chloride Sodium carbonate	Rosettes and sheaves of needles spreading to large size. Rosettes of transparent plates. Spherical crystals, but not needles as in cinchonine.

*Characteristics of Microchemical Tests for Alkaloids—Continued*

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Cinchonine (132)	Sodium carbonate. Disodium phosphate	Dark rosettes, composed of radiating needles, form immediately. Similar to crystals formed by sodium carbonate, but more burr-shaped.
Cocaine (133)	Platinic chloride	Delicate, feathery crystals; later becoming heavier in structure.
Codeine (133)	Marmé Wagner	Silvery, circular masses, crystallizing into dark rosettes of irregular outline. Heavy, red-brown precipitate; crystallizes very slowly in yellow blades extending in branches (never red).
Cotarnine (134)	Platinic chloride Mercuric chloride Potassium ferrocyanide	1:200. Hair-like crystals, yellow and curving. Colorless, long, branching needles. Acidified with 1 drop of 5% HCl; globules that develop into dense, burr-shaped crystals; also amber-brown plates.
Dilaudid (135)	Sodium nitroprusside	To minute quantity (<1 mg) in 2 drops H <sub>2</sub> O add minute fragment of reagent. Elongated 6-sided prisms; also in aggregates.
Ephedrine (136)	Kraut	Long, brown radiating and interlacing needles.
Ethylhydrocupreine (135) (Optochin)	Ammonium thiocyanate	1:100 in 0.1 N HCl. Long, straight needles.
Ethylmorphine (138) (Dionine)	Wagner Mercuric chloride	1:200. Groups of yellow needles, branching later. Transparent plates often with notched ends; singly and in groups. Stir to start crystallization.
Heroine (139)	Platinic chloride	Spherical clusters of golden yellow needles form slowly around a nucleus; cluster disintegrates on standing.
Homatropine (140)	Gold chloride	1:200. Green-gold blades, often with pointed ends and united in pairs; surfaces appear etched on long standing.
Hydrastine (141)	One drop of 5% HCl and potassium ferrocyanide	1:100. Spheres of radiating crystals. Shake slide to start crystallization. Avoid excess reagent.
Hydrastinine (142)	Potassium permanganate Mercuric chloride One drop of 5% hydrochloric acid and potassium ferrocyanide	1:500. Immediate red plates, often with serrated edges. In concentrated soln, great number of large red or brown plates with deeply cut edges. 1:500. Transparent needles forming branches rapidly in neutral and acidified solns. 1:200. Yellow rhombic plates and tree-like crystals.

*Characteristics of Microchemical Tests for Alkaloids—Continued*

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Hyoscyamine (143)	Gold chloride	Thin, transparent, nearly colorless irregular plates, often curved. Crystals form slowly in 1:100 to 1:200 soln. Shaking the slide aids crystallization.
Morphine (144)	Marmé Wagner	Silvery, gelatinous precipitate, crystallizing in dense masses of fine needles. Small drop of reagent produces heavy, red-brown precipitate, slowly crystallizing in shining, red, overlapping plates extending in branches.
Narceine (145)	Wagner, or zinc potassium iodide Platinic chloride	1:400. Blue, radiating needles, sometimes with yellow dichroism. Beautiful, feathery rosettes develop in all solns.
Narcotine (145)	Potassium hydroxide or ammonium hydroxide	1:200. White amorphous precipitate, which crystallizes slowly; dense rosettes of needles.
Nicotine (146)	Mercuric chloride  Mercuric chloride-sodium chloride	Radiating transparent blades form in presence of slight excess of $H_2SO_4$ ; feather-like blades form in presence of HCl. Radiating transparent blades.
Papaverine (147)	Zinc chloride	Thin rectangular plates in excess HCl.
Physostigmine (148)	Lead iodide Gold bromide	1:100. Radiating serrated plates. 1 mg. in 1 drop $H_2O$ . Brown dendritic aggregates.
Pilocarpine (149)	Platinic chloride	Crystals form slowly; layers of thin yellow, triangular plates of delicate structure.
Procaine hydrochloride (150)	Platinic chloride Gold chloride and hydrochloric acid	Spherical crystals of radiating branches. Irregular, radiating branches.
Quinidine (151)	Potassium iodide	Small, triangular crystals in great numbers; best in 1:1000 dilution; soluble in excess reagent.
Quinine (151)	Disodium phosphate	Silvery, sheaf-like crystals.
Scopolamine (152) (Hyoscine)	Gold chloride	Clusters of pale yellow, transparent blades, with coarse, saw-toothed edges form immediately on shaking the slide. Crystals grow to large size in 1:200 soln.
Sparteine (153)	Gold chloride	Large numbers of blade-like crystals varying in size according to concentration.
Stovaine (154)	To soln add drop of HCl and gold chloride	1:50. Tree-like crystals.

*Characteristics of Microchemical Tests for Alkaloids—Continued*

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Strychnine (155)	Platinic chloride Marmé	Crystals form immediately in clusters and singly in small, wedge-shaped needles, which move about the field. Silvery masses, slowly forming rosettes.
Theobromine (156)	Kraut (freshly prepared)	In hydrochloric acid (1+3). Tufts of brown radiating needles form readily in 1:200 soln.
Theophylline (156)	Ammoniacal silver nitrate Mercuric chloride	1:200. Gelatinous at first; dense spheres of dark radiating needles. 1:150. Spheres and double tufts of dense radiating needles.
Yohimbine (157)	Sodium carbonate	In 1:1000 soln heated to 50°. Fine needles in sheaf-like bundles and rosettes.

## MICROCHEMICAL TESTS FOR SYNTHETICS—OFFICIAL

## 39.245

## REAGENTS

- (a) *Acetic acid*.—Dilute 6 ml of acetic acid to 100 ml with H<sub>2</sub>O.
- (b) *Ammoniacal nickel acetate soln*.—Mix 1 volume of 5% Ni(C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>)<sub>2</sub>·4H<sub>2</sub>O with 1 volume of 10% NH<sub>3</sub>. Use clear supernatant liquid.
- (c) *Ammoniacal silver nitrate soln*.—See 39.241(a).
- (d) *Ammonium thiocyanate soln*.—See 39.241(c).
- (e) *Barium hydroxide soln*.—Saturated soln in H<sub>2</sub>O.
- (f) *Benzaldehyde*.—N.F.VII quality.
- (g) *Bromide-bromate soln*.—Dissolve 0.3 g of KBrO<sub>3</sub> and 1.2 g of KBr in H<sub>2</sub>O and dilute to 100 ml.
- (h) *Gold bromide soln*.—See 39.241(e).
- (i) *Gold chloride soln*.—See 39.241(f).
- (j) *Kraut*.—See 39.241(h).
- (k) *Lead acetate soln*.—Dissolve 5 g of Pb(C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>)<sub>2</sub>·3H<sub>2</sub>O in H<sub>2</sub>O and dilute to 100 ml.
- (l) *Lead triethanolamine soln*.—Add 1 ml of triethanolamine (tech. 90% is satisfactory) to soln of 1 g of Pb(C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>)<sub>2</sub>·3H<sub>2</sub>O in 20 ml of H<sub>2</sub>O. Slight turbidity does not interfere.
- (m) *Magnesia mixture*.—Dissolve 55 g of MgCl<sub>2</sub>·6H<sub>2</sub>O and 140 g of NH<sub>4</sub>Cl in H<sub>2</sub>O. Add 130.5 ml of NH<sub>4</sub>OH and dilute to 1 liter with H<sub>2</sub>O.
- (n) *Marmé*.—See 39.241(j).
- (o) *Mercuric chloride soln*.—See 39.241(k).
- (p) *Mercurous nitrate soln*.—Dissolve 15 g of HgNO<sub>3</sub>·H<sub>2</sub>O in mixture of 90 ml of H<sub>2</sub>O and 10 ml of 10% HNO<sub>3</sub>. Preserve in dark, amber-colored bottle containing small globule of Hg.
- (q) *Nitric acid*.—1+1.
- (r) *Phosphotungstic acid soln*.—Dissolve 5 g of P<sub>2</sub>O<sub>5</sub>·24WO<sub>3</sub>·xH<sub>2</sub>O in 100 ml of H<sub>2</sub>O.
- (s) *Picric acid*.—Crystals.
- (t) *Picrolonic acid soln*.—Dissolve 250 mg of 1-(p-nitrophenyl)-3-methyl-4-nitropyrrolone in 25 ml of alcohol.
- (u) *Platinic chloride soln*.—See 39.241(m).
- (v) *Potassium ferrocyanide soln*.—See 39.241(n).

(w) *Silicotungstic acid soln.*—Dissolve 5 g of  $4\text{H}_2\text{O} \cdot \text{SiO}_2 \cdot 12\text{WO}_3 \cdot 22\text{H}_2\text{O}$  in 100 ml of ca 6 N  $\text{H}_2\text{SO}_4$ .

(x) *Silver nitrate soln.*—Dissolve 1 g of  $\text{AgNO}_3$  in 20 ml of  $\text{H}_2\text{O}$ .

(y) *Sodium nitrite soln.*—Dissolve 10 g of  $\text{NaNO}_2$  in  $\text{H}_2\text{O}$  and dilute to 100 ml.

(z) *Wagner.*—See 39.241(w).

(aa) *Zinc pyridine soln.*—Add 1 ml of pyridine to soln of 1 g of  $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$  in 20 ml of  $\text{H}_2\text{O}$ .

### 39.246 *Characteristics of Microchemical Tests for Synthetics*

SYNTHETIC	SOLVENT	CONCENTRATION OF SYNTHETIC	REAGENT	DESCRIPTION OF TESTS AND CRYSTALS
Acetanilid (158)	10% HCl	1:100	Phosphotungstic acid	Rosettes of prisms.
	10% HCl	1:100	Bromide-bromate soln	Small prisms.
Acetophenetidin (158)		About 1 mg of powdered material	Nitric acid	After adding a drop of nitric acid let stand for a few seconds, then add a drop of $\text{H}_2\text{O}$ . Bright yellow, curving, branched crystals.
	10% HCl	Saturated soln	Wagner	Large, irregular plates.
Acetylsalicylic acid (159)	2% triethanolamine	1:50	Silver nitrate	Fine, curling, hair-like crystals form first near edge of the drop.
Aminopyrine (160)	$\text{H}_2\text{O}$	1:100	Mercuric chloride Marmé	Long, slender radiating crystals, often curved. Groups of spiny branches.
Amytal (161)	3% $\text{NH}_4\text{OH}$	1:50	Acetic acid	Long branching needles; some hexagonal plates in groups.
	3% $\text{NH}_4\text{OH}$	1:25	Acetic acid	Groups of rectangular plates.
Antipyrine (162)	$\text{H}_2\text{O}$	1:100	Potassium ferrocyanide	Acicular and prismatic crystals form after a drop of 1% HCl is added.
Barbital (163)	—	Ca 1 mg of powder	Ammoniacal silver nitrate	Stir to aid solution and crystallization. Very small twinned crystals and larger tufts.
	3% $\text{NH}_4\text{OH}$	1:50	Acetic acid	Dark burrs (stirring hastens crystallization).
Benzoic acid (164)	—	Dry powder	Lead triethanolamine	Stir a small quantity of the synthetic into a drop of reagent. Stir thoroly to induce crystallization. 4-sided plates, singly and in groups.
	—	Dry powder	Zinc pyridine	Stir a small quantity of synthetic into a drop of reagent. Stir thoroly to induce crystallization. Hexagonal crystals.
	2% triethanolamine	1:100 to 1:200	Silver nitrate	Rods or curving blades with irregular ends.

*Characteristics of Microchemical Tests for Synthetics—Continued*

SYNTHETIC	SOLVENT	CONCENTRATION OF SYNTHETIC	REAGENT	DESCRIPTION OF TESTS AND CRYSTALS
Cinchophen (166)	0.1 N NaOH Add H <sub>2</sub> O, and make slightly acid with HCl	1:1000	Gold chloride	Dark clusters of needles. Few short, rhombic crystals.
Diallyl- barbituric acid (166)	—	Dry powder	Lead tri- ethanol- amine	Stir a small quantity of the synthetic into a drop of the reagent. Rods singly and in clusters.
	—	Dry powder	Barium hy- droxide	Stir a small quantity of the synthetic into a drop of the reagent. Rods singly and in groups.
Dinitro- phenol (167)	Small quan- tity of 0.1 N NaOH	1:100	Hydro- chloric acid	Plates with four branches. In more dilute soln single, rectangular plates.
Hydroxy- quinoline sulfate (168) (Chinosol)	Dissolve the salt in H <sub>2</sub> O. If free base, dissolve in 10% HCl, avoiding excess	1:500	Magnesia mixture	Small, elliptical grains. Few burr-shaped crys- tals on standing.
Mandelic acid (169)	H <sub>2</sub> O	1:100	Lead acetate	Rosettes of thin curving plates.
	H <sub>2</sub> O	1:100	Mercurous nitrate	Burr-shaped groups of needles.
Methen- amine (170)	H <sub>2</sub> O	1:500	Silicotung- stic acid	Thin transparent, rec- tangular crystals.
Metrazol (171)	H <sub>2</sub> O	—	Mercuric chloride (1:10)	Rods, many almost needle-like; frequently in groups; also in radi- ating aggregates.
	H <sub>2</sub> O	1:100	Silicotung- stic acid	Amorphous; changes to elongated prisms; also long needles.
Neocinchophen (172)	10% HCl	Saturated soln	Ammonium thiocyanate	Rosettes of needles. (Gentle agitation by tip- ping the slide back and forth hastens crystalliza- tion.)
	10% HCl	Saturated soln	Platinic chloride	Needles in clusters.
Pheno- barbital (173)	—	About 1 mg of powder	Ammoniacal nickel acetate	Stir to aid solution and crystallization. Single rectangular crystals.
Pyridium (174)	Dissolve the salt in H <sub>2</sub> O. If free base, dissolve in 10% HCl, avoiding excess	1:1000	Ammonium thiocyanate	Small, red-brown dense sheaves.

*Characteristics of Microchemical Tests for Synthetics—Continued*

SYNTHETIC	SOLVENT	CONCENTRATION OF SYNTHETIC	REAGENT	DESCRIPTION OF TESTS AND CRYSTALS
Salicyclic acid (175)	10% HCl	Dry powder	Bromide-bromate soln	Stir a few crystals of the synthetic into a drop of the HCl. Add a drop of the bromide-bromate soln. Fine needles appear to grow from the crystals of salicylic acid.
	—	Dry powder	Lead triethanol-amine	Stir a few crystals into a drop of the reagent. Rods or needles grow from the crystal of salicylic acid.
	2% triethanol-amine	1:100 to 1:200	Silver nitrate	Small irregular plates; a few short rods.
Sulfadiazine (176)	H <sub>2</sub> O	—	Gold bromide	Red circular masses composed of fine needles.
Sulfanilamide (177)	—	Dry powder	Benzaldehyde	Stir thoroly a small amount of synthetic into a drop of reagent. 4-sided plates. Yellow needles.
	0.1 N HCl	Saturated soln	Sodium nitrite	
Sulfapyridine (178)	Acetone + H <sub>2</sub> O	—	Gold chloride	Yellow rods or blades, also X-shaped aggregates.
Sodium sulfapyridine monohydrate (178)	H <sub>2</sub> O	1:100	Gold chloride	Yellow rods in X-shaped aggregates.
Sulfathiazole (179)	50% alcohol	—	Picric acid	Long, fine yellow needles, many curved, occur in dense rosettes; also short stout rods in groups or singly.
	50% alcohol (or no solvent)	—	Picrolonic acid	Distinct rosettes of very fine needles; also single needles.
Triethanolamine (180)	H <sub>2</sub> O	1:100	Kraut	Oily globules changing to large, red, hexagonal plates and prismatic crystals.

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- (53) Ibid., 3, 379 (1920); 4, 573 (1921); 6, 269 (1923).
- (54) Ibid., 4, 156, 416 (1920-1); 8, 547 (1925); 9, 53 (1926); 19, 105 (1936).
- (55) Ibid., 19, 105, 534 (1936); 28, 85 (1945).
- (56) Ibid., 21, 555 (1938); 22, 94, 729 (1939); 28, 85 (1945).
- (57) Ibid., 20, 82, 577, 631 (1937); 27, 112 (1944); 28, 85 (1945).
- (58) Ibid., 14, 311 (1931); 15, 407 (1932); 16, 82, 343 (1933); 22, 100 (1939).
- (59) Ibid., 12, 276 (1929); 13, 310 (1930); 15, 406 (1932); 16, 81, 342 (1933).
- (60) Ibid., 12, 291 (1929); 13, 329 (1930); 14, 83, 327 (1931).
- (61) Ibid., 18, 552 (1935); 19, 528 (1936); 20, 588 (1937); 22, 92, 726 (1939).
- (62) Ibid., 8, 529 (1925); 9, 301 (1926); 10, 359 (1927); 11, 50, 339 (1928).
- (63) Ibid., 15, 448 (1932); 16, 84, 375 (1933); 23, 90 (1940); 28, 85 (1945).
- (64) Ibid., 15, 452 (1932); 16, 375 (1933); 18, 89, 555 (1935).



- (65) *Ibid.*, 15, 83, 442 (1932).  
 (66) *Ibid.*, 12, 300 (1929); 13, 333 (1930); 14, 83, 337 (1931).  
 (67) *Ibid.*, 12, 296 (1929); 14, 52, 330 (1931).  
 (68) *Ibid.*, 13, 332 (1930); 14, 330 (1931); 15, 81, 418 (1932).  
 (69) U. S. Dept. Agr. Bur. Chem. Circ. 94, p. 1; J. Ind. Eng. Chem., 4, 374 (1912); J. Assoc. Official Agr. Chem., 28, 85 (1945).  
 (70) J. Assoc. Official Agr. Chem., 19, 535 (1936); 21, 557 (1938); 22, 95, 730 (1939).  
 (71) *Ibid.*, 8, 526 (1925); 9, 296 (1926); 10, 45 (1927); 11, 335 (1928); 12, 50 (1929); 14, 53, 360 (1931); 22, 99, 761 (1939); 28, 85 (1945).  
 (72) *Ibid.*, 16, 383 (1933); 17, 78, 451 (1934); 18, 84, 519 (1935); 27, 111 (1944).  
 (73) *Ibid.*, 10, 383 (1927); 11, 360 (1928); 12, 288 (1929); 13, 326 (1930); 16, 348 (1933); 17, 52, 440 (1934); 18, 532 (1935).  
 (74) *Ibid.*, 21, 95, 554 (1938); 28, 85 (1945).  
 (75) *Ibid.*, 20, 83, 589 (1937); 28, 85 (1945).  
 (76) *Ibid.*, 18, 464 (1935); 20, 82, 592 (1937); 22, 100 (1939).  
 (77) *Ibid.*, 15, 136 (1932); 21, 543 (1938); 22, 100, 622 (1939).  
 (78) *Ibid.*, 16, 384 (1933); 20, 81, 564 (1937).  
 (79) *Ibid.*, 22, 98, 757 (1939); 27, 111 (1944); 28, 85 (1945).  
 (80) *Ibid.*, 12, 49, 261 (1929).  
 (81) *Ibid.*, 25, 107, 839 (1942).  
 (82) J. Ind. Eng. Chem., 12, 766 (1920); J. Assoc. Official Agr. Chem., 7, 14, 271 (1923-4); 8, 30, 541 (1924); 22, 99 (1939); 28, 85 (1945).  
 (83) *Ibid.*, 8, 541 (1925); 25, 843 (1942); 26, 311 (1943); 27, 111 (1944).  
 (84) N. F. VII, 322; Ind. Eng. Chem., Anal. Ed., 14, 53 (1942); J. Assoc. Official Agr. Chem., 27, 343 (1944); 28, 85 (1945).  
 (85) J. Assoc. Official Agr. Chem., 20, 576 (1937); 21, 94, 552 (1938); 28, 85 (1945).  
 (86) *Ibid.*, 27, 111, 360 (1944).  
 (87) *Ibid.*, 25, 524 (1942); 26, 238 (1943); 27, 111 (1944).  
 (88) *Ibid.*, 22, 97, 748 (1939); 27, 112 (1944).  
 (89) *Ibid.*, 14, 51, 321 (1931).  
 (90) *Ibid.*, 18, 87, 526 (1935).  
 (91) *Ibid.*, 19, 104, 517 (1936).  
 (92) *Ibid.*, 10, 44, 343 (1927).  
 (93) *Ibid.*, 11, 48, 326 (1928).  
 (94) *Ibid.*, 49.  
 (95) *Ibid.*, 15, 84, 422 (1932); 27, 111 (1944).  
 (96) *Ibid.*, 15, 456 (1932); 16, 379 (1933); 17, 75, 425 (1934); 27, 111 (1944); 28, 85 (1945).  
 (97) *Ibid.*, 18, 87, 525 (1935); 19, 516 (1936); 20, 555 (1937); 21, 529 (1938); 22, 712 (1939); 27, 111 (1944).  
 (98) *Ibid.*, 22, 90, 712 (1939); 23, 59 (1940); 27, 111 (1944).  
 (99) *Ibid.*, 15, 83, 419 (1932); 27, 111 (1944).  
 (100) *Ibid.*, 24, 93, 833 (1941); 28, 85 (1945).  
 (101) *Ibid.*, 24, 54, 93, 833 (1941).  
 (102) *Ibid.*, 28, 86 (1945).  
 (103) *Ibid.*, 27, 357 (1944); 28, 86 (1945).  
 (104) *Ibid.*, 14, 84, 370 (1931); 22, 100 (1939); 23, 59 (1940).  
 (105) *Ibid.*, 15, 50, 85, 434 (1932); 22, 100 (1939).  
 (106) *Ibid.*, 15, 50, 85, 441 (1932); 22, 100 (1939); 23, 59 (1940).  
 (107) *Ibid.*, 10, 367 (1927); 11, 51, 343 (1928); 12, 52 (1929); 19, 106 (1936).  
 (108) *Ibid.*, 14, 82, 312 (1931); 23, 59, 90 (1940); 27, 111 (1944).  
 (109) *Ibid.*, 12, 52, 280 (1929); 28, 85 (1945).  
 (110) *Ibid.*, 17, 75, 432 (1934).  
 (111) *Ibid.*, 22, 96, 743 (1939); 28, 85 (1945).  
 (112) *Ibid.*, 18, 85, 520 (1935); 28, 85 (1945).  
 (113) *Ibid.*, 17, 462 (1934); 18, 89, 544 (1935); 28, 85 (1945).  
 (114) *Ibid.*, 13, 364 (1930); 14, 351 (1931); 16, 83, 292, 364 (1933); 22, 100 (1939); 23, 59 (1940).  
 (115) *Ibid.*, 21, 96, 571 (1938); 27, 111 (1944); 28, 85 (1945).  
 (116) *Ibid.*, 11, 53, 355 (1928).  
 (117) *Ibid.*, 8, 269, 551 (1925); 9, 54, 312 (1926); 10, 46, 69 (1927).  
 (118) *Ibid.*, 22, 96, 739 (1939); 28, 85 (1945).  
 (119) *Ibid.*, 13, 334 (1930); 14, 53, 341 (1931); 27, 111 (1944); 28, 85 (1945).

- (120) Ibid., 10, 383 (1927); 11, 53, 362 (1928); 14, 51, 325 (1931).
- (121) Ibid., 14, 51, 325 (1931).
- (122) Ibid., 15, 81, 413 (1932).
- (123) Ibid., 20, 79, 551 (1937); 21, 92, 525 (1938).
- (124) Ibid., 15, 81, 413 (1932).
- (125) Ibid., 11, 354 (1928); 12, 54, 282 (1929); 14, 51, 317 (1931).
- (126) Ibid., 25, 105, 830 (1942).
- (127) Ibid., 16, 84 (1933); 22, 90 (1939); 23, 89 (1940).
- (128) Ibid., 21, 91, 525 (1938).
- (129) Ibid., 22, 88, 706 (1939); 28, 85 (1945).
- (130) Ibid., 13, 46, 315 (1930).
- (131) Ibid., 26, 96 (1943).
- (132) Ibid., 12, 53, 282 (1929).
- (133) Ibid., 10, 372 (1927); 11, 52, 353 (1928).
- (134) Ibid., 22, 88, 706 (1939); 28, 85 (1945).
- (135) Ibid., 24, 92, 830 (1941).
- (136) Ibid., 14, 83, 317 (1931).
- (137) Ibid., 20, 80, 553 (1937).
- (138) Ibid., 21, 91, 525 (1938).
- (139) Ibid., 5, 154 (1921); 7, 6, 273 (1924); 10, 370 (1927); 11, 354 (1928).
- (140) Ibid., 18, 85, 521 (1935).
- (141) Ibid., 20, 79, 551 (1937); 21, 92, 525 (1938).
- (142) Ibid., 21, 92, 525 (1938).
- (143) Ibid., 18, 86, 521 (1935).
- (144) Ibid., 10, 372 (1927); 11, 52, 354 (1928).
- (145) Ibid., 22, 88, 706 (1939); 28, 85 (1945).
- (146) Ibid., 16, 82, 345 (1933).
- (147) Ibid., 17, 76, 433 (1934).
- (148) Ibid., 23, 89, 746 (1940); 24, 92, 830 (1941); 28, 85 (1945).
- (149) Ibid., 11, 354 (1928); 12, 54, 282 (1929); 14, 82, 317 (1931).
- (150) Ibid., 17, 76, 433 (1934).
- (151) Ibid., 12, 53, 282 (1929).
- (152) Ibid., 18, 86, 521 (1935).
- (153) Ibid., 16, 82, 345 (1933).
- (154) Ibid., 23, 89, 746 (1940); 28, 85 (1945).
- (155) Ibid., 11, 52, 353 (1928).
- (156) Ibid., 19, 102, 512 (1936).
- (157) Ibid., 15, 81, 413 (1932).
- (158) Ibid., 19, 103, 514 (1936); 22, 90 (1939); 23, 89 (1940).
- (159) Ibid., 21, 93, 528 (1938); 22, 90 (1939); 23, 89 (1940).
- (160) Ibid., 18, 86, 523 (1935); 22, 90 (1939); 23, 89 (1940).
- (161) Ibid., 20, 80, 553 (1937); 22, 90 (1939); 23, 89 (1940).
- (162) Ibid., 17, 77, 435 (1934); 22, 90 (1939); 23, 89 (1940).
- (163) Ibid., 20, 80, 553 (1937); 22, 90 (1939); 23, 89 (1940).
- (164) Ibid., 21, 93, 528 (1938); 22, 90 (1939); 23, 89 (1940).
- (165) Ibid., 16, 84, 391 (1933); 22, 90 (1939); 23, 89 (1940).
- (166) Ibid., 22, 90, 709 (1939).
- (167) Ibid., 18, 86, 523 (1935); 20, 90 (1939); 23, 89 (1940).
- (168) Ibid., 16, 84, 391 (1933); 22, 90 (1939); 23, 89 (1940).
- (169) Ibid., 22, 89, 709 (1939).
- (170) Ibid., 17, 77, 435 (1934); 22, 90 (1939); 23, 89 (1940).
- (171) Ibid., 25, 106, 830 (1942); 28, 85 (1945).
- (172) Ibid., 19, 103, 514 (1936); 22, 90 (1939); 23, 89 (1940).
- (173) Ibid., 20, 81, 553 (1937); 22, 90 (1939); 23, 89 (1940).
- (174) Ibid., 16, 84, 391 (1933); 22, 90 (1939); 23, 89 (1940).
- (175) Ibid., 21, 93, 528 (1938); 22, 90 (1939); 23, 89 (1940).
- (176) Ibid., 26, 96 (1943); 27, 110 (1944); 28, 85 (1945).
- (177) Ibid., 22, 89, 709 (1939); 27, 112 (1944); 28, 85 (1945).
- (178) Ibid., 24, 92, 830 (1941); 28, 85 (1945).
- (179) Ibid., 25, 106, 830 (1942); 28, 85 (1945).
- (180) Ibid., 17, 77, 435 (1934); 22, 90 (1939); 23, 89 (1940).

## 40. MICROBIOLOGICAL METHODS

### EXAMINATION OF EGGS AND EGG PRODUCTS (1)—OFFICIAL, FIRST ACTION

The "Manual of Methods for Pure Culture Study of Bacteria" of the Society of American Bacteriologists should be used as a guide for the further study of micro-organisms obtained in the cultural procedures described.

#### SAMPLING

##### 40.1

#### EQUIPMENT

(a) *Liquid eggs*.—Sampling tube or dipper, sterile sample containers with tight closures (pint Mason jars or friction top cans most practical), alcohol, alcohol lamp or other burner, absorbent cotton, clean cloth or towel, water pail.

(b) *Frozen eggs*.—Electric (high speed) or hand drill with 1×16" auger, hammer and steel strip (12×2×0.25") or other tool for opening cans, tablespoon, hatchet or chisel, precooled sterile containers etc. as listed under (a).

(c) *Dried eggs*.—Grain trier of sufficient length to reach to bottom of containers to be sampled. Clean sample containers with tight closures (pint Mason jars or paperboard cartons), clean cloth or towel, tablespoon.

##### 40.2

#### PROCEDURE

Secure samples from representative number of containers in lot, 23.1. Sterilize sampling tube or dipper, auger, spoon, and hatchet by wiping with alcohol-soaked cotton and flaming over alcohol lamp or other burner. Between each sample wash instrument thoroly, dry, and resterilize. (The grain trier and spoon used in sampling dried eggs need not be sterilized but should be wiped with a clean cloth after each sampling.) Open and sample all containers under as nearly aseptic conditions as possible.

(a) *Liquid eggs*.—Mix contents of container thoroly with sterile sample tube or dipper and transfer ca  $\frac{1}{2}$  pint to sterile sample container. Maintain samples below 5° but avoid freezing. Observe and record odor of each container sampled as normal, putrid, sour, or musty.

(b) *Frozen eggs*.—Remove top layer of egg with sterilized hatchet or chisel. Drill three cores from top to bottom of container: one core in center, a second midway between center and periphery, and a third core near edge of container. Transfer drillings from container to sample container with sterile spoon. Examine product organoleptically by smelling at opening of a fourth drill-hole made after removal of bacteriological sample. (Heat produced by the electric drill intensifies odor of egg material, thus facilitating organoleptic examination.) Record odors as normal, putrid, sour, or musty. Refrigerate samples with dry ice (solid CO<sub>2</sub>) or other suitable refrigerant if analysis is to be delayed or sampling point is at some distance from laboratory.

(c) *Dried eggs*.—For small packages, take entire parcel or parcels for the sample. For boxes and barrels, remove top layer with spoon or other clean instrument and with clean trier remove at least 3 cores as directed under (b). (Samples should consist of ca  $\frac{1}{2}$  pint.) Transfer core aseptically to sample container by means of clean spoon or other suitable instrument. Store samples under refrigeration or in cool place.

## ANALYTICAL PROCEDURE

## 40.3

## PREPARATION OF SAMPLE

(a) *Liquid eggs*.—Thoroughly mix sample with sterile spoon or sterile electric stirrer before analysis. Prepare 1–10 dilution by aseptically weighing 11 g of egg material into sterile, wide-mouth, glass or rubber-stoppered bottle; add 99 g of sterile physiological NaCl soln and 1 tablespoonful of glass shot. Agitate the 1–10 dilution thoroughly to insure complete soln or distribution of egg material in diluent, by shaking container rapidly 100 times, each shake being an up-and-down excursion of ca 1 foot, time interval not exceeding 45 seconds. Prepare serial dilutions from 1–100 to 1–100,000,000 as needed, using physiological saline soln. Proceed as directed under 40.4–40.9(a). Pour all plates and inoculate other media within 15 min. after preparation of first dilution in order to prevent growth or death of microorganisms in the dilutions, thus affecting final results.

(b) *Frozen eggs*.—Thaw frozen egg material as rapidly as possible in order to prevent increase in numbers of microorganisms present and at temp. sufficiently low to prevent destruction of the microorganisms (37°). (Frequent rotary shaking of the sample container aids in thawing the frozen material. Thawing temp. may be maintained by use of water bath or bacteriological incubator.) Proceed as directed under (a).

(c) *Dried eggs*.—Thoroughly mix sample with sterile spoon or spatula. Prepare 1–10 dilution as directed under (a). If material is poorly soluble in physiological saline (stored samples) use 0.1 N LiOH as diluent. Prepare serial dilutions as directed under (a) and proceed as directed under 40.4–40.9(b).

## 40.4

## PLATE COUNTS

Inoculate one set of Petri plates with 1 ml portion of all dilutions (1–10 to 1–1,000,000 as needed). Pour plates with nutrient agar previously cooled to 42–45°. Incubate inoculated plates at 32° for 3 days. Count plates with the aid of Quebec colony counter, if available. Express final results as numbers of viable microorganisms/g of egg material.

## 40.5

## INCIDENCE OF COLIFORM GROUP

(a) *Presumptive test*.—Inoculate 1.0 ml portions from suitable dilutions (1–10 to 1–100,000,000) of egg material into fermentation tubes of lactose broth. Incubate at 37° for 24–48 hours. Streak eosin methylene blue or Endo agar plates from all lactose broth cultures showing gas production. Incubate plates at 37° for 24–48 hours. Examine plates of differential media for colonies of microorganisms of coliform group. Record number of coliform bacteria/g of egg material as reciprocal of highest dilution showing positive confirmation on differential media.

(b) *Completed test*.—Inoculate from colonies of coliform types of bacteria appearing on differential agar plates to nutrient agar slants. Incubate at 37° for 24 hours. Purify cultures for further study. Obtain biochemical reactions of purified cultures by following tests:

*Kovac test*: Indol production;

*Methyl red (M. R.) and Voges Proskauer (V. P.) tests*;

*Koser sodium citrate test*: Utilization of Na citrate as sole source of C.

NOTE: Follow procedure for biochemical reactions recommended in "Standard Methods of Water Analysis," 8th ed., 1936, of American Public Health Association.

**40.6 INCIDENCE OF HEMOLYTIC STAPHYLOCOCCI AND STREPTOCOCCI**

Inoculate Petri plates with 1 ml portions of all dilutions from 1-100 to 1-1,000,000. Pour plates with veal-infusion agar containing 6% of defibrinated horse, sheep, or rabbit blood (0.6 ml of blood/10 ml of medium). Cool agar to 40° and add blood just prior to pouring plates. Incubate plates for 24 hours at 37°. Confirm presence of coccus types of microorganisms by microscopic examination of smears taken from representative colonies and stained by Gram's method. Express final results as numbers/g.

**40.7 TESTS FOR PUTREFACTIVE ANAEROBIC TYPES OF MICROORGANISMS**

Exhaust tubes of Holman cooked meat medium, 40.11(d), before use, by heating in streaming steam for 15 min., followed by rapid cooling in water bath. Inoculate tubes of exhausted meat medium with 1 ml portions of all dilutions from 1-10 to 1-100,000. Incubate inoculated tubes at 37° for 3-4 days. (Putrefactive anaerobes are evidenced by evolution of gas and digestion of meat.) Confirm cultures by microscopic examination of smears stained with Gram stain. Record numbers of putrefactive anaerobes/g of egg material as reciprocal of highest dilution showing positive anaerobic growth.

**40.8 TESTS FOR FUNGI**

Inoculate Petri plates with 1 ml portions of all serial dilutions from 1-10 to 1-100,000. Acidify melted Bacto-malt agar to pH 3.5 with 85% U.S.P. lactic acid as directed on the package label for Bacto-malt agar. Pour inoculated plates with acidified malt agar previously cooled to 42-45°. Incubate plates for 5 days at 20° or at room temp., if a 20° incubator is not available. Express final results as number of fungi/g of egg material. Confirm yeast colonies by microscopic examination of smears stained by Gram method.

**40.9 DIRECT MICROSCOPIC COUNTS**

*North aniline oil methylene blue stain.*—Mix 3.0 ml of aniline oil with 10.0 ml of alcohol, and add 1.5 ml of HCl slowly with constant agitation. Add slowly 30.0 ml of a saturated alcoholic methylene blue soln, then make to volume of 100.0 ml with H<sub>2</sub>O and filter.

(a) *Liquid and frozen eggs.*—Place 0.01 ml of undiluted egg material on clean dry microscopic slide and spread over area of 1 sq. cm. (circular area with diam. of 1.13 cm preferred). Permit the smear preparation to dry on level surface at 35-40°. Immerse in xylene for minimum of 1 min., then immerse in alcohol for minimum of 1 min. Stain for 45 seconds in the North aniline oil methylene blue. Wash by repeated immersions in H<sub>2</sub>O and dry thoroly before examination. Observe subsequent procedure and precaution as directed in "Standard Methods for the Examination of Dairy Products," 8th Ed., 1941, of the American Public Health Association. Express final results as number of bacteria/g of egg material.

(b) *Dried eggs.*—Place 0.01 ml of the 1-10 or 1-100 dilution of dried egg material on clean dry microscopic slide and spread over 2 sq. cm.

**NOTE:** 0.1 N LiOH may be used as diluent and is preferred for samples that are poorly soluble in physiological saline. A circular area with diameter of 1.6 cm. is preferable. Addition of a drop of H<sub>2</sub>O to each smear will facilitate uniform spreading.

Proceed as directed under (a). Double the microscopic factor, since an area of 2 sq. cm. is used, and multiply the count by 10 or 100, depending upon whether smear was prepared from 1-10 or 1-100 dilution.

## CULTURE MEDIA

## 40.10

## STANDARD METHODS MEDIA

Prepare following media as recommended in "Standard Methods of Water Analysis," 8th Ed., 1936, of the American Public Health Association: Nutrient agar, Levine eosin methylene blue agar, Endo agar, tryptophane broth, lactose broth, methyl red—Voges Proskauer peptone medium, and Koser Na citrate medium.

## 40.11

## OTHER MEDIA

(a) *Malt agar*.—Malt extract (Difco), 3% or 30.0 g; agar, 1.5% or 15.0 g; and H<sub>2</sub>O, 1000 ml. Boil to dissolve medium. Sterilize at 15 lbs. pressure for 20 min. Final pH 5.5.

NOTE: For detection of fungi in egg products, malt agar should be acidified to pH 3.5 with 85% lactic acid U.S.P. after agar has been melted and prior to use. Medium should not be reheated after addition of acid.

(b) *Physiological salt soln.*—NaCl, C.P., 0.85% = 8.5 g; and H<sub>2</sub>O, 1000 ml. Sterilize mixture at 15 lbs. pressure for 15 min.

(c) *Veal infusion agar*.—Ground lean veal, 500.0 g, and H<sub>2</sub>O, 1000 ml.

Infuse overnight in refrigerator and strain thru cheesecloth without pressure. Make up to original volume with H<sub>2</sub>O and skim off any fat. Steam in Arnold 30 min.; and filter thru paper. Add peptone (Difco), 1.0% or 10.0 g; NaCl, 0.5% or 5.0 g; and agar, 1.5% or 15.0 g.

Steam in Arnold to dissolve ingredients. Adjust reaction to pH 7.6 and steam in Arnold 15 min. Filter thru Büchner funnel with paper pulp mat, by aid of suction. (Use egg albumin for clarification when necessary. Add fresh white of 1 egg previously beaten with 50 ml of the medium or its equivalent in desiccated egg white (1.5 g) to each liter of the medium before adjustment of reaction and after cooling to 50°. Shake thoroly to insure soln of egg white. Allow to stand 20 min. Heat in Arnold for 15 min. to coagulate egg white. Shake vigorously and reheat. Filter. Adjust reaction to pH 7.6. Steam in Arnold 15 min. Filter.) Distribute 10 ml quantities into test tubes or 80 ml quantities into bottles. Sterilize at 15 lbs. pressure (121°) for 20 min. Final pH 7.4. For hemolytic tests cool melted agar to 40° and add 6% of defibrinated horse, sheep, or rabbit blood prior to pouring plates (0.6 ml of blood/10 ml of medium).

(d) *Holman cooked meat medium (alkaline)*.—Ground fresh lean beef, 500.0 g; H<sub>2</sub>O, 1000 ml; peptone (Bacto), 5.0 g; and NaCl, C.P., 5.0 g.

Add beef to the H<sub>2</sub>O and infuse overnight in refrigerator. Skim off fat. Strain thru several layers of cheesecloth and press out broth, retaining meat press cake. Make up to original volume of 1 liter. Add peptone and heat in Arnold 10 min. Filter and add NaCl. Add normal NaOH until alkaline to phenolphthalein. Heat in Arnold 15 min. to clear, and filter. Distribute pressed-out beef remaining from infusion into test tubes (150×20 mm), ca 2 g into each tube, and add 10 ml of the clear alkaline broth. Sterilize in autoclave at 15 lbs. pressure (121°) for 20 min. Final reaction pH 7.2–7.4. Prior to use, boil tubed medium at least 10 min. to expel absorbed O and cool promptly in water bath.

DETECTING AND ESTIMATING NUMBERS OF THERMOPHILIC  
BACTERIA IN SUGAR (§)—OFFICIAL, FIRST ACTION

(Sugar, both beet and cane, may carry spores of all three groups of thermophilic bacteria that are important as spoilage agents in non-acid canned foods, i.e., flat sour bacteria (*Bacillus stearothermophilus*), thermophilic anaerobes not producing

hydrogen sulfide (*Clostridium thermosaccharolyticum*), and sulfide spoilage bacteria (*Cl. nigrificans*). These bacteria are economically the most important causes of spoilage thru understerilization of non-acid canned vegetables.)

## 40.12

## SAMPLING

Take  $\frac{1}{2}$  lb. samples from each of 5 bags or barrels of shipment or of lot in question. Send these samples to laboratory in clean sealed cans, or other appropriate containers.

(Adequacy of this sampling will vary in relation to size of shipment or lot, and if there is any significant variability in the shipment, this fact will become evident, in majority of cases, thru individual tests on 5 samples.)

## 40.13

## PREPARATION OF SAMPLE

Place 20 g of sugar in sterile 150 ml Erlenmeyer flask marked to indicate volume of 100 ml. Add sterile  $H_2O$  to 100 ml mark. Bring rapidly to boiling, and boil 5 min. Replace evaporation with sterile  $H_2O$ .

## 40.14

## CULTURE MEDIA

(a) *Dextrose tryptone agar*.—For use in detection of "flat-sour" bacteria.

This medium is prepared as a standardized, dehydrated medium and is marketed under the name of Bacto-dextrose Tryptone Agar by the Difco Laboratories, Inc., Detroit, Mich. Because of its standardization, its use in this form is recommended. It may, however, be prepared according to following formula: Tryptone, 10 g; dextrose, 5 g; agar, 15 g; bromocresol purple, 0.04 g; and  $H_2O$ , 1000 ml.

(b) *Liver broth*.—For detection of thermophilic anaerobes not producing  $H_2S$  (*Cl. thermosaccharolyticum*), putrefactive anaerobes, and other mesophilic anaerobes.

Mix chopped beef liver with  $H_2O$  in proportion of 500 g to 1000 ml. Boil mixture slowly 1 hour, adjust to ca pH 7.0, and boil an additional 10 min. Then press boiled material thru cheese cloth and make liquid to 1000 ml. To broth add 10 g of peptone and 1 g of  $K_2HPO_4$ . Adjust reaction to pH 7.0. In tubing, introduce  $\frac{1}{2}$ –1" of previously boiled ground beef liver into the tube.

Before using, unless it is freshly prepared, exhaust medium by subjecting to streaming steam at least 20 min., and after inoculation, stratify it with 2–2 $\frac{1}{2}$ " layer of plain nutrient agar (common formula), which has been cooled to 50°.

(c) *Sulfite agar (modified)*.—For detection of thermophilic anaerobes producing  $H_2S$  (*Cl. nigrificans*).

Prepare according to following formula: Tryptone, 10 g;  $Na_2SO_3$ , 1 g; agar, 20 g; and  $H_2O$ , 1000 ml.

At time of tubing place clean iron strip or nail in tube. No adjustment in reaction is necessary. Prepare medium at frequent intervals (1 week), and if  $Na_2SO_3$  is used in soln also prepare it at frequent intervals (1 week).

## CULTURE TECHNIC

## 40.15

## DETECTION OF FLAT SOUR SPORES

Into each of 5 Petri dishes pipet 2 ml of the boiled sugar soln. Cover, and mix inoculum with the dextrose tryptone agar. Incubate plates at 55° for 36–48 hours, and to prevent drying of the agar, humidify the incubator. The combined count from the 5 plates represents the number of spores in 2 g of original sugar. Multiply this count by 5 to express results in terms of number of spores/10 g of sugar.

These colonies are characteristic. A colony is round, measures 2-5 mm in diam., presents a typical opaque central "spot," and is usually surrounded by a yellow halo in a field of purple. This halo may be insignificant, or missing, where certain low acid-producing types are concerned, or where the plate is so thickly seeded that the entire plate takes on a yellow tinge. The typical subsurface colonies are rather compact and may approach the "pin point" condition.

If there is doubt as to the identity of the sub-surface colonies, a decision can usually be made after observing the nature of the surface colonies. If they show reasonable purity of flora, it is safe for practical purposes to assume that the sub-surface colonies have been formed by similar bacterial groups. It is emphasized that where the plate is heavily seeded, there may be loss of accuracy as regards counts, and colony structure and size may be atypical. If plates are so heavily seeded as to make counting impracticable, a second sample of the sugar may be plated, dilutions of the original soln being used.

Whether atypical subsurface colonies are flat sour organisms may often be determined by streaking from the colonies to agar plates so that their surface characteristics may be noted.

No immediate significance is attached to the presence of "non-spoilage" thermophiles; i.e., aerobic spore-formers, actinomyces, etc., although when present in large numbers they carry significance with regard to the general bacteriological quality of the sugar. The total thermophilic spore count may be obtained from the dextrose tryptone agar plates.

#### 40.16 DETECTION OF THERMOPHILIC ANAEROBES NOT PRODUCING $H_2S$

(Under the conditions stated, thermophilic anaerobes are manifest thru the splitting of agar and the presence of acid. At times a cheesy odor is noted. The method is considered suitable as a qualitative test but quantitatively it provides only a means of rough estimation. The method does not permit expression of results in terms of numbers of spores per unit weight of sugar.)

Divide 20 ml of the sugar soln ca equally among 6 liver broth tubes and stratify the liquid medium with plain nutrient or yeast water agar. After agar has solidified, preheat to 55° and incubate at that temp. for 72 hours.

#### 40.17 DETECTION OF THE THERMOPHILIC ANAEROBES PRODUCING $H_2S$ (SULFIDE SPOILAGE ORGANISMS)

(In sulfite agar the sulfide spoilage organisms are detected thru the formation of characteristic blackened spherical areas. Due to the solubility of  $H_2S$  and its fixation by the Fe, no gas is noted. Certain of the thermophilic anaerobes (not producing  $H_2S$ ), methods for the detection of which precede, give rise to relatively large quantities of  $H_2$  which splits the agar and reduces the sulfite, thereby causing general blackening of the medium. This condition, however, is readily distinguishable from the restricted blackened areas mentioned previously. The blackened areas may be counted to obtain quantitative results.)

Divide 20 ml of the sugar soln ca equally among 6 freshly exhausted tubes containing the sulfite agar. Incubate at 55° for 72 hours.

#### 40.18 REPORTING RESULTS

Report flat sour and sulfide spoilage results as number of spores/10 g of sugar. Report thermophilic anaerobes (not producing  $H_2S$ ) as number of tubes positive and number negative in the following manner: + + + - - -.



## EXAMINATION OF CANNED VEGETABLES (5)—OFFICIAL, FIRST ACTION

### 40.19

### SAMPLING

The procedure to be followed in the microbiological examination of canned vegetables is indicated by the purpose of the examination. Samples are commonly submitted to the laboratory for one of following three purposes:

1. Unspoiled samples—for direct bacteriological examination for sterility.
2. Unspoiled samples—for examination as to keeping quality.
3. Spoiled samples—for examination as to cause of spoilage.

The technic of examination for all three types of samples is similar with respect to treatment of container, removal of samples, and culture methods. Differences in treatment include the following:

(a) Before being cultured, unspoiled samples submitted for examination as to keeping quality should be incubated at 37.5° for 1 month. This time of incubation, necessarily an arbitrary matter, is considered the longest period that is practicable in the usual case. Anaerobes, which at times may remain dormant for many months, may escape detection, but usually the likelihood of spoilage in the product under ordinary commercial conditions of handling is indicated in this time. With canned vegetables it is frequently necessary to incubate at 55° in order to determine possibility of thermophilic spoilage in event of undercooling following processing. At 55° incubation for 10 days is recommended.

(b) Samples submitted for examination for cause of spoilage should be given direct microscopic examination in order to obtain general information regarding the bacterial flora. Ordinary laboratory stains, such as carbol fuchsin or gentian violet, are suitable in preparing mounts. The Gram stain is not recommended. A Gram negative result would be of little significance because of lack of knowledge regarding age of bacteria in spoiled material.

(a) *Physical examination and preparation of can.*—(1) Note and record all marks of identification, either embossed on can or appearing on label.

(2) Remove labels. Record any physical defects such as rustiness, pin-holing, dents, improper closure, or defective side seams. Plainly mark for inspection questionable points if can is to be pumped or given any other physical examination after it is opened.

(3) Clean container with soap and H<sub>2</sub>O; if greasy, it may be found helpful, especially at site of opening, to apply a suitable solvent, such as petroleum benzine, alcohol, or naphtha.

(4) For sterilization at site of opening, preferably grasp container in the hand and hold previously cleaned top in flame of a Bunsen burner, distributing heat with circular motion. Do not play burner down upon top of can because this will result in a concentration of heat at top, causing scorching of material, and it might lead to spurling of contents when opening is made. (Such sterilization also causes a release of vacuum in can, which will prevent any contamination that might result from an inrush of air when the opening is made.) When containers are badly swollen (4), for safety preferably sterilize with HgCl<sub>2</sub> soln (1+1000) for a few seconds, dry with sterile towel, and sample without flaming; or thoroly clean cans with 60% alcohol. Whichever later treatment is used, first thoroly cleanse cans with soap and H<sub>2</sub>O, as it is possible that neither the HgCl<sub>2</sub> soln nor the alcohol treatment would insure complete destruction of spore contaminants in the time that elapses between the sterilization treatment and the opening of the container.

(b) *Removal of sample.*—(1) *Opening of container.*—After flaming, or otherwise sterilizing the point of opening, make aperture with appropriate type of opener, which has also previously been sterilized by flaming. (Openers of spiral or circular type are preferred.) With liquid products, puncture an opening with a sharpened instrument of appropriate diameter (5).

(2) *Inoculum*.—Determine type of instrument to be used for removal of inoculum by character of the food under examination. Sample liquid or semi-liquid food products with sterile unf tapered pipets or inverted 10 ml pipets. Sample solid material with sterile cork borers or brass sampling tubes after they have been wrapped in paper and sterilized 30 min. at 15 lbs. pressure in autoclave. Stopper samplers with cotton plugs, and force solid material into culture tubes by means of sterile glass rod or some similar device. Take a sample of at least 15 g of food material, which may be cultured directly into 1 culture tube or flask, but preferably into at least 3 culture vessels. If the material is solid, mix it with sterile  $H_2O$  as preliminary step to inoculation.

## 40.20

## CULTURE MEDIA (8)

For routine culture purposes, dextrose tryptone agar and liver broth are suitable for the detection of those organisms that are the principal cause of spoilage in non-acid canned vegetables, as well as for the detection of less important organisms occasionally encountered, and use of these media is recommended. Where special examination is made for putrefactive anaerobes, beef heart peptic digest is recommended to supplement liver broth cultures. Corn-liver medium should supplement liver broth in special search for thermophilic anaerobes or in their study after isolation. Sulfite agar is useful only when indicated by a type of spoilage characterized by the presence of  $H_2S$ .

(a) *Dextrose tryptone agar*.—See 40.14(a).

This medium is used principally for the isolation of flat sour bacteria from original products or from enrichment cultures. It is also suitable for the isolation of other aerobic or facultative anaerobic bacteria, such as may be encountered in non-acid canned foods. For flat sour bacteria, incubation is usually at 55°.

(b) *Liver broth*.—See 40.14(b).

In this medium, thermophilic anaerobes are evident thru the splitting of agar and the presence of acid. At times, a cheesy odor is noted. When incubation is at 37°, the presence of putrefactive anaerobes becomes apparent thru splitting of the agar, resulting from gas production, and the presence of a putrid odor.

(c) *Beef heart peptic digest broth* (7).—Used principally for detection of putrefactive anaerobes and their cultural study.

This medium is difficult to prepare, but if any intensive study of putrefactive spoilage is made, it is regarded as a valuable medium.

(1) Slowly heat to boiling 1000 g of finely ground, fat-free heart, and 1000 ml of tap  $H_2O$ , and adjust to reaction of pH 8.0–8.2. Cool, and carefully skim off layer of fat that floats on the cold medium. To each liter of beef heart mash, add 2 liters of peptic digest broth (2). Adjust reaction to pH 7.2–7.4.

(2) Wash clean and mince finely 5 or more large pig stomachs. Mince an equal amount of clean pig or beef liver. Mix in following proportions: Minced pig stomachs, 400 g; minced liver, 400 g; HCl, 40 g; and tap  $H_2O$  at 50°, 4000 g.

Keep mixture in glass or porcelain receptacles for 18–24 hours. Make biuret and also tryptophan tests. When both reactions are positive, the digest is green-yellowish and contains little undigested debris. Transfer digest to large bottles and steam 10 min. at 100° to stop digestion. Strain digest thru cotton or preferably store overnight in ice chest and decant after 24 hours. Warm decanted digest to 70° and neutralize with  $Na_2CO_3$  (twice normal soln) to litmus at this temp. Filter desired quantity, add 0.2%  $K_2HPO_4$ ; adjust to pH 7.4; and mix with beef heart mash. Adjust final reaction and sterilize 1 hour at 18 lbs. pressure. Incubate for 5 days and repeat same sterilization for 1 hour at 18 lbs. pressure.

Before inoculation, exhaust as directed in 40.14(b). After inoculation, stratify with sterile vaseline.

(d) *Corn-liver medium (8)*.—For detection of thermophilic anaerobes not producing  $H_2S$  (*Cl. thermosaccharolyticum*), putrefactive anaerobes, and other mesophilic anaerobes.

For a time it is suggested that this medium be used in conjunction with another anaerobic medium such as liver broth, in order that comparative data may be obtained.

Steam 1 or 2% liver (tissue from liver infusion medium dried at 55–60° and finely ground) and 5% corn meal for 1 hour, cool, and tube. Carefully sterilize the resulting rather viscous medium by autoclaving 2 hours at 15–17 lbs. pressure. If pressure is reduced slowly after sterilization, short (6") tubes may be used without blowing the plugs. This medium need not be steamed just before using, requires no seal nor incubation in an anaerobic jar, and satisfactory results may be obtained with 2–5 cm depth of medium.

Positive cultures are recognized by the appearance of gas with or without digestion of the medium. There may also be a measurable change in the reaction of the medium.

(e) *Sulfite agar*.—For detection of thermophilic anaerobes producing  $H_2S$  (*Cl. nigrificans*).

Sulfide spoilage organisms are detected thru formation of characteristic blackened spherical areas. Usually no gas forms. The presence of gas coupled with general blackening of medium indicates presence of thermophilic anaerobes not of sulfide spoilage group. This darkening results from reduction by  $H$  gas.

Use following formula: Water, 1 liter; tryptone, 10 g;  $Na_2SO_3$ , 1 g; and agar, 20 g.

At time of tubing place clean Fe strip or nail in tube. No adjustment in reaction is necessary.

#### 40.21

#### ROUTINE EXAMINATIONS

Incubate original cultures at 37.5° for 48–72 hours.

### EXAMINATION OF CANNED FRUITS (9) AND OTHER ACID CANNED FOODS—TENTATIVE

#### 40.22

#### SAMPLING

The three primary objectives sought in the microbiological examination of canned foods that come within the pH range of "acid products" are the following:

- (1) Detection of spoilage.
- (2) Determination of commercial soundness (keeping quality).
- (3) Determination of sterility and the detection of type of bacteria capable of causing spoilage when the product is utilized as a sauce with other non-acid type foods.

The procedures for all three objectives, with respect to treatment of container, removal of sample, quantity of inoculum, and cultural methods are essentially the same. Modifications in treatment include the following:

(a) Incubate normal appearing cans submitted for examination for commercial soundness or keeping quality at 30° if less than 14 days has elapsed since the product was packed. Additional incubation at this temp. to insure at least 14 days' incubation is desirable. When no 30° incubator is available, incubation at an average room temp. of 25° may suffice.

(b) Incubate samples examined for the presence of other than spoilage organisms at 37° in culture media suitable for the detection of organisms capable of causing spoilage of non-acid foods. Products such as tomato pastes, purées, and ketchups are often used as packing media (sauces) for non-acid products and may be responsible for spoilage under these changed conditions of pH.

(c) For samples submitted for examination for cause of spoilage include direct microscopic examination as provided under 40.19(b).

(d) Determination of pH is useful to supplement data for the detection of spoilage.

(a) *Physical examination and preparation of can.*—See 40.19(a).

(b) *Removal of sample.*—(1) *Opening of container.*—See 40.19(b)(1).

(2) *Inoculum.*—Sample liquid or semi-liquid food products with sterile untapered pipets or inverted tapered pipets of suitable capacity. (The untapered pipets should have minimum dimensions of 350 mm in length and 5 mm inside diameter.) Sample solid or semi-solid food products with sterile spatulas, long-handled spoons, or other instruments (10), depending on character of food under examination. Use pipets only for products of such viscosity as to permit transfer of inoculum into culture media by gravity. Use a sample consisting of a minimum of 15 g or 15 ml of the food material and divide it into aliquots for duplicate culturing in each of different culture media used in examination.

(3) *Sampling of product fractions.*—When representative inocula are desired from both liquid and solid parts, transfer solid component of sample to culture medium with forceps sterilized by flaming and use culture media in large test tubes (100×25 mm) or in wide-mouth jars or bottles.

#### 40.23

#### CULTURE MEDIA (11)

Because of their acid nature many products in this class are subjected to the limited processing designed only to prevent spoilage. This is done to preserve the color, flavor, and texture of the foods. In some instances the products are filled into the cans while hot and receive no further heat processing. Two main groups of microorganisms encountered in the spoilage of this class of canned food products are the aciduric bacteria and the yeasts. Viable bacteria capable of producing spoilage in non-acid foods but rendered inactive in acid foods have frequently been responsible for the reporting of false positive results in examinations for spoilage in acid canned foods. Use following media adjusted to reaction below pH 5.0 for detection of spoilage organisms:

1. *Aciduric spoilage bacteria.*—

(a) *Buffered acid meat medium*

	grams
Distilled water.....	1000 ml
Ground fresh lean beef.....	500
Proteose peptone.....	5
Sodium chloride.....	5
Dextrose.....	10
Potassium citrate.....	12
Citric acid.....	11

Infuse beef-water mixture overnight in refrigerator. Heat in Arnold or boil for 30 min. Strain thru several layers of cheese cloth and press out broth, retaining meat press cake. Add H<sub>2</sub>O to infusion to make to 1 liter. Add the peptone and heat in Arnold or boil 10 min. Filter, and add salt. Acidify with K citrate and citric acid to pH 4.6, add the dextrose, and filter. Distribute the pressed-out beef remaining from infusion into medium sized test tubes (150×20 mm), ca 2 g into each tube, and add 10 ml of the broth. Sterilize in autoclave at 15 lbs. pressure for 15 min. Prior to using, boil the tubed medium for 10 min. to expel absorbed O and cool promptly in a water bath.

The preparation of plate cultures is optional, and when desirable, the following plating medium is suitable:

(b) *Digest-yeast-tomato juice*

Tryptic casein digest (dehydrated).....	20 g
Bacto yeast extract.....	2 g
Tomato juice.....	100 ml
Dextrose.....	10 g
Agar.....	15 g
Distilled water to make.....	1000 ml

Dissolve by boiling, or heating, in an Arnold sterilizer, adding the tomato juice before sterilization. Sterilize in autoclave for 15 min. at 15 lbs. pressure.

2. *Bacteria inhibited below pH 5.0.*—Use a medium essentially the same as the acid meat medium with reaction adjusted to pH 7.2, omitting the K citrate-citric acid mixture. (This medium, when rid of excess free O by boiling and prompt cooling just prior to use, has been found satisfactory for the growth of anaerobes, aerobes, and facultative bacteria.)

3. *Yeast spoilage.*—(a) *Clarified malt extract medium*

Dry malt extract (Difco)....	100 g
Distilled water.....	1000 ml

Dissolve the powdered malt extract in the H<sub>2</sub>O by heating in Arnold sterilizer, or on water bath. Adjust to pH 4.7 and cool to 50°. Add slowly 100 ml of a 5% suspension of Bentonite (colloidal clay) and mix vigorously. Hold at 50–75° for 30 min., then filter thru a fluted-paper filter until clear. Heat filtrate in autoclave 10 min. at 15 lbs. pressure, and filter thru paper to remove any precipitate formed. Distribute into tubes, or flasks. For a plating medium, dissolve by heating 2% agar in the clarified broth, and filter if necessary thru cotton and cheese cloth. To avoid further precipitation sterilize at 10 lbs. pressure for 15 min. and cool promptly. (Bacto Malt Extract Broth can now be obtained in convenient dehydrated form and may be substituted for the above medium.)

4. *"Flat-sour" spoilage bacteria.*—See 40.14(a).

## 40.24

## INCUBATION

Incubate all cultures for the detection of spoilage organisms for at least 72 hours at 30°. For the detection of non-aciduric bacteria, incubate at 37° for at least 48 hours. For the occasional "flat-sour" spoilage encountered in such products as tomato juice, incubate for thermophilic anaerobes at 56° for at least 48 hours.

## 40.25

## CULTURE STUDY

Use the "Manual of Methods for Pure Culture Study of Bacteria" of the Society of American Bacteriologists as a guide for study of microorganisms obtained in the cultural procedure described.

## EXAMINATION OF NUTS AND NUT PRODUCTS (12) TENTATIVE

## 40.26

## PREPARATION OF SAMPLE

Place in sterile 125 ml bottle, fitted with a ground-glass stopper, 50 g of nut meats, comprising particles selected at random from sample or subsample. Use strict aseptic precautions in opening the package, carton, jar, or other receptacle holding the sample, and in transferring meats to sterile bottle use a metal spoon sterilized by immersion in alcohol followed by heating by direct flame.

Add to nut meats 50 ml (or 50 g) of sterile  $H_2O$ . Shake vigorously 3 min., or longer if necessary to effect a thoro washing of the nut meats. Following this agitation, allow specimen to stand ca 5 min., again shake vigorously, and withdraw quantities of the suspension for direct inoculation of culture media and for the preparation of serial dilutions.

## 40.27

## CULTURE TECHNIC

Transfer to series of fermentation tubes of lactose broth 2 ml portions of original washings and of 1:10 and 1:100 dilutions of the washings. (In view of dilution originally effected by washing 50 g of nut meats in 50 ml of  $H_2O$ , a 2 ml inoculum is required in each case to permit interpretation of results in terms of washings of 1 g, 0.1 g, and 0.01 g of nut meats.) Incubate lactose broth cultures 24 hours at 37° and examine for gas production.

Inoculate a series of plates with 2 ml portions of 1:100 and 1:1000 dilutions of the washings. Pour plates with nutrient agar and incubate for 48 hours at 37°. Determine total aerobic counts and express as numbers per g of nut meats.

From the lactose broth cultures showing gas, streak plates poured with Levine eosin-methylene-blue agar or with Endo agar. Incubate plates 24 hours at 37°. Examine the eosin-methylene blue or Endo agar plates for colonies of organisms of the coliform group. Select representative colonies for transfer to nutrient agar slants. Incubate the slant cultures for 24 hours at 37°. Purify cultures for further study. Ascertain the biochemical reactions of the purified cultures by the following tests: *Kovac Test*: Indol production; *Methyl Red (M.R.)* and *Voges-Proskauer (V.P.) Tests*; *Koser Na Citrate Test*: Utilization of Na citrate as sole source of C.

Follow the procedures recommended in "Standard Methods of Water Analysis," 8th edition (1936), of the American Public Health Association, under Appendix I, Section XVII (Differentiation—Coli-Aerogenes Group Organisms).

The biochemical types encountered usually fall within the following six classes:

<i>Indol</i>	<i>M.R.</i>	<i>V.P.</i>	<i>Citrate</i>	<i>Type</i>
+	+	—	—	<i>Escherichia coli</i>
—	+	—	—	<i>E. coli</i> (atypical)
+	+	—	+	<i>E. freundii</i> (coli intermediate)
—	+	—	+	<i>E. freundii</i> (atypical coli intermediate)
—	—	+	+	<i>Aerobacter aerogenes</i>
+	—	+	+	<i>A. aerogenes</i> (atypical)

Coliform organisms giving other sets of reactions may occasionally be encountered.

## SELECTED REFERENCES

- (1) J. Assoc. Official Agr. Chem., 22, 625 (1939).
- (2) *Ibid.*, 19, 439 (1936); 21, 457 (1938).
- (3) *Ibid.*, 21, 452 (1938).
- (4) *Ibid.*, 19, 430 (1936).
- (5) *Ibid.*, 431.
- (6) *Ibid.*, 433.
- (7) J. Infectious Diseases, 31, 505 (1922).
- (8) J. Bact., 28, 267 (1934).
- (9) J. Assoc. Official Agr. Chem. 21, 454 (1938); 24, 95 (1941).
- (10) *Ibid.*, 19, 431 (1936).
- (11) *Ibid.*, 440.
- (12) *Ibid.*, 25, 109 (1942).

## 41. MICROCHEMICAL METHODS

### METHOXYL AND ETHOXYL GROUPS (I)—TENTATIVE

#### 41.1

#### REAGENTS

(a) *Acetic acid-potassium acetate soln.*—Dissolve 10 g of K acetate in sufficient acetic acid to make 100 ml of soln.

(b) *Sodium acetate soln.*—Dissolve 25 g of  $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$  in sufficient  $\text{H}_2\text{O}$  to make 100 ml of soln.

(c) *Approximately 0.05 N thio-sulfate soln.*—Boil 2.5 liters of  $\text{H}_2\text{O}$  until  $\frac{1}{2}$  has evaporated, cool to ca  $75^\circ$ , and then add the necessary  $\text{Na}_2\text{S}_2\text{O}_3$  and 20 ml of amyl alcohol (by-product from alcoholic fermentation). Allow to cool and standardize against a standard  $\text{KIO}_3$  soln.

#### 41.2 DETERMINATION

To 5 ml of the K acetate soln, add 0.2 ml of  $\text{Br}_2$ , and place  $\frac{1}{2}$  of this liquid in receiver C and the remainder in D (Fig. 68). Then weigh ca 20 mg of substance upon a tared piece of cigaret paper ( $15 \times 25$  mm) and place both paper and contents in bottom of boiling flask A, together with a boiling rod. (A glass tube ca 60 mm long, 3.5 mm o.s. diameter with a 1 mm bore. It is sealed at one end and also closed ca 10 mm from the other. The open end is fire polished. When this rod is placed in flask with open end down it will cause uniform boiling indefinitely if sufficient heat is constantly applied to flask.) Add 2.5 ml of melted phenol from a wide-tipped pipet and 5 ml of  $\text{HI}$ , 41.3(c), and connect flask to remainder of apparatus, which consists of the trap (B), containing a little  $\text{H}_2\text{O}$ , and the receivers C and D. Pass  $\text{CO}_2$  thru apparatus from capillary side arm of boiling flask at uniform rate of 15 ml per min., and boil liquid by means of a mantled micro burner at such rate that vapors of boiling liquid rise about half way in air condenser. Continue boiling 30–60 min. (If

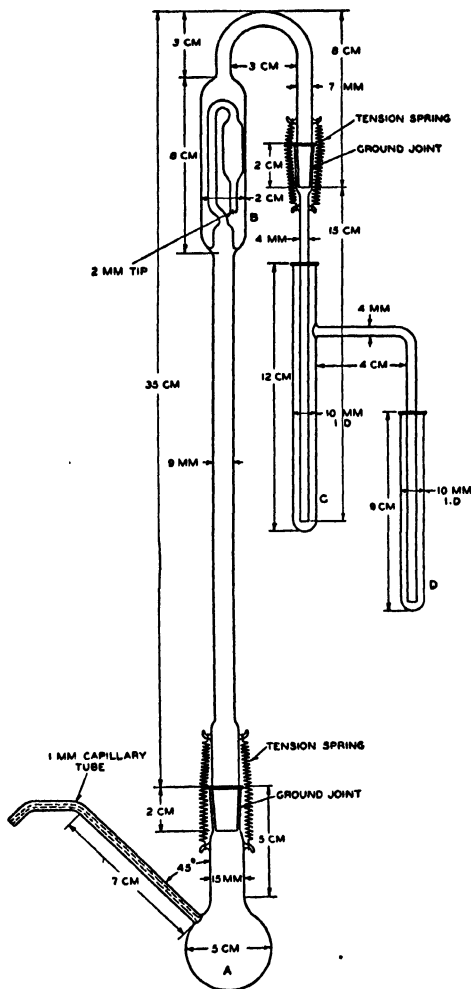


FIG. 68.—SEMI-MICRO ZEISEL METHOXYL APPARATUS

consists of the trap (B), containing a little  $\text{H}_2\text{O}$ , and the receivers C and D. Pass  $\text{CO}_2$  thru apparatus from capillary side arm of boiling flask at uniform rate of 15 ml per min., and boil liquid by means of a mantled micro burner at such rate that vapors of boiling liquid rise about half way in air condenser. Continue boiling 30–60 min. (If

substance is of type known to require only 30 min., this period should be used, but material about which such information is lacking should be boiled one hour.) Disconnect apparatus and wash contents of receivers into 250 ml Erlenmeyer flask containing 5 ml of Na acetate soln. Adjust volume of liquid to 100 ml and reduce excess  $\text{Br}_2$  with formic acid (ca 15 drops is sufficient).

Remove any  $\text{Br}_2$  vapor in flask by drawing air over liquid from vacuum line or by blowing air over liquid, then add 0.5 g of KI and 5 ml of 10%  $\text{H}_2\text{SO}_4$ . Titrate liberated I with the thiosulfate soln, using starch as indicator.

Obtain blank on all reagents by making a determination without a sample and subtract this from quantity of  $\text{Na}_2\text{S}_2\text{O}_3$  soln used when sample was present. 1 ml of 0.05 N  $\text{Na}_2\text{S}_2\text{O}_3 = 0.2586$  mg of methoxyl ( $\text{OCH}_3$ ).

The same procedure applies to ethoxyl groups. 1 ml of 0.05 N  $\text{Na}_2\text{S}_2\text{O}_3 = 0.3754$  mg of ethoxyl ( $\text{OC}_2\text{H}_5$ ). For determination of higher alkoxyls the air condenser and scrubber may be surrounded by a water jacket heated to necessary temperature.

## NITROGEN

### *Microkjeldahl Method (2)—Tentative*

#### 41.3

##### REAGENTS

(a) *Methyl red*.—0.1% alcoholic soln.

(b) *Sodium hydroxide-sodium thiosulfate soln*.—Aqueous soln of 40 g of NaOH and 5 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ /100 ml.

(c) *Constant boiling hydriodic acid*.—N-free. Heat to  $50^\circ$  a mixture of 254 g of I and 185 ml of  $\text{H}_2\text{O}$  in 500 ml flask fitted with ground-joint condenser and dropping funnel. Add portionwise 60 g of 50% hypophosphorous acid at such a rate that mixture gently boils. When I is reduced, apply heat and reflux the acid soln 3 hours while a liberal stream of  $\text{CO}_2$  passes thru the soln. Change condenser to allow distillation and collect the constant boiling HI. Add 2 ml of 50%  $\text{H}_3\text{PO}_4$  and store in dark glass-stoppered bottles.

#### 41.4

##### OPERATION OF DISTILLATION APPARATUS (3)

Generate steam in 1 (Fig. 70) by resistance coil immersed in  $\text{H}_2\text{O}$ . (Quantity of steam delivered is controlled, preferably, by variable transformer or sliding rheostat.) Close 3 with haemostatic forceps and 4 by removing funnel from wire hook and allowing it to hang so as to crimp the rubber connection. (Steam then passes thru trap 2 and distilling flask 5, and is condensed in 7 and collected in 6.) When the distillation is completed, remove spent liquor in 5 by breaking heating current. As soon as liquid in 5 is transferred to 2, remake heating current, open 3 to allow liquid in 2 to pass to waste and add  $\text{H}_2\text{O}$  to 5 thru 4. Close 3 and 4 again and repeat operation, which rinses 5, thus conditioning apparatus for next distillation.

#### 41.5

##### DETERMINATION

Place in Kjeldahl flask, dimensions of which are shown in Fig. 69, ca 10 mg of substance, weighed upon  $15 \times 25$  mm piece of cigaret paper; 40 mg of  $\text{HgO}$ , 0.5 g of  $\text{K}_2\text{SO}_4$ , and 1.5 ml of  $\text{H}_2\text{SO}_4$ . Gently heat flask and contents upon digester until frothing ceases, then increase temp. until acid mixture boils vigorously and vapors of acid rise to within 5 cm of mouth of flask. (Time from beginning to end of digestion should be 1 hour, and mixture should be colorless during the last 30 min. A longer combustion period does no harm.) Cool digest, add 1 drop of alcohol, and again heat mixture until it is colorless. (When acid mixture has cooled it is ready for distillation.)



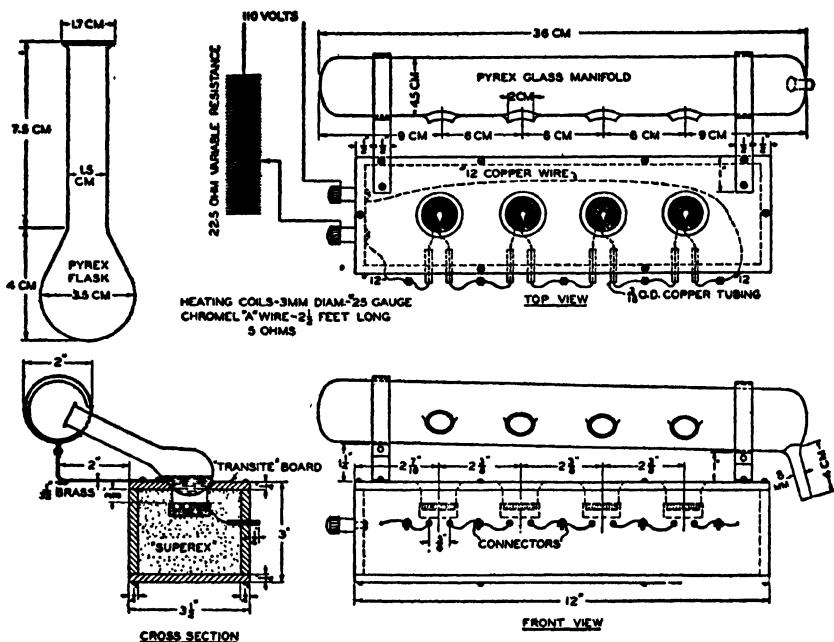


FIG. 69.—MICROKJELDAHL DIGESTER

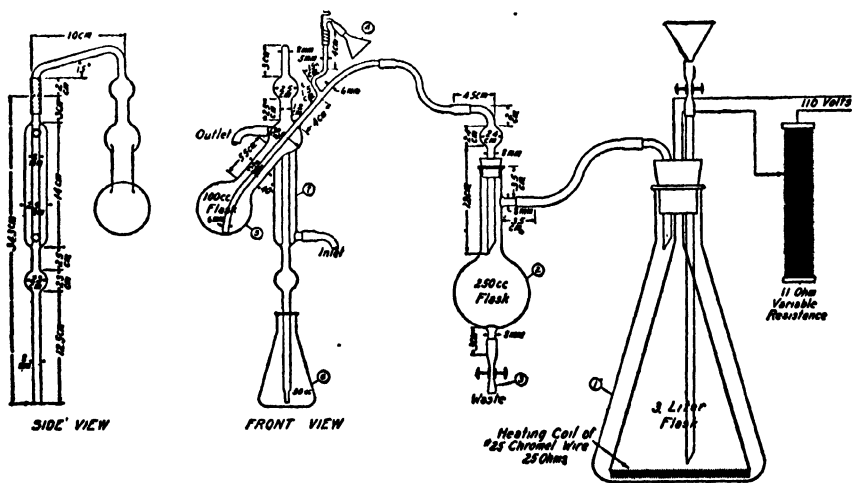


FIG. 70.—MODIFIED PARMAS-WAGNER MICROKJELDAHL DISTILLING APPARATUS

Pass steam thru distilling apparatus for some time to assure proper working condition, then empty. Restart steam generator, open 3 and 4, and close rubber connection between 2 and 5 with haemostatic forceps. Dilute acid mixture in digestion flask with ca 8 ml of  $H_2O$ . Cover lip of digestion flask with thin film of vaseline to prevent any liquid running down outside of flask, and transfer soln to 5 thru 4. Rinse flask with four 3 ml portions of  $H_2O$  to make transfer quantitative. Next add sufficient of the  $NaOH-Na_2S_2O_3$  soln thru 4 to neutralize acid and render final liquid strongly alkaline and wash it into distilling flask with 2 or 3 ml of  $H_2O$ .

Remove funnel from its support and allow to hang so as to crimp the rubber and thus close system at this point. Close 3, open connection between 2 and 5, and place a small flame under 5. (Distillation begins almost immediately.) Collect condensate containing the  $NH_3$  in flask 6, containing 2 ml of 4%  $H_3BO_3$  soln, to which is added 1 drop of the methyl red indicator.

Continue distillation with adapter dipping in the acid soln until 8 ml of distillate is collected. Lower flask until adapter is above contents of flask, and in this position continue distillation until ca 1 ml more of distillate is collected. During this time wash outside of adapter with a little  $H_2O$  in fine stream from wash bottle. (During these operations so adjust rate of distillation that none of liquid in 5 is mechanically carried over, and that at end of distillation temp. of contents of receiving flask does not exceed  $40^\circ$ .) Titrate the  $NH_3$  in the  $H_3BO_3$  soln with 0.02 N HCl, using buret graduated to 0.05 ml. Determine blank due to reagents, and after subtracting this value from buret reading calculate percentage of N in sample as follows:

$$\frac{(0.28) (\text{ml of } 0.02 \text{ N HCl used}) (100)}{\text{Weight of sample}} = \% \text{ N.}$$

## 41.6

*Friedrich Method (4)—Tentative*(For N-N, NO and  $NO_2$  linkages)

Weigh upon a piece of cigaret paper, as indicated in 41.5, ca 10 mg of substance and place in digestion flask. Add 1 ml of the constant-boiling HI, 41.3(c), and gently reflux mixture for 45 min. Next apply more heat so that ca 0.7 ml of the HI slowly distills from the flask. Remove flask from digester and add 0.5 g of  $K_2SO_4$ , 1 ml of  $H_2O$ , and 1.5 ml of  $H_2SO_4$ . Heat mixture on digester until most of the  $H_2O$  is removed. Cool digest, add another ml of  $H_2O$ , and repeat distillation, the purpose being to remove the liberated I with steam; if this is not accomplished with 2 ml of  $H_2O$ , add another ml and repeat process. Cool digest, add 40 mg of  $HgO$ , and complete digestion as directed under 41.5.

## SELECTED REFERENCES

- (1) J. Am. Chem. Soc., 51, 1479 (1920); J. Assoc. Official Agr. Chem., 15, 136 (1932); 20, 292 (1937); 22, 100, 622 (1939).
- (2) Ibid., 16, 255 (1933); 24, 97, 641, 648 (1941).
- (3) Biochem. Z., 125, 253 (1921).
- (4) Z. Physiol. Chem., 216, 68 (1933).

## 42. EXTRANEOUS MATERIALS IN FOODS AND DRUGS\*

### 42.1

#### APPARATUS

(a) *Wildman trap flask* (1) (Fig. 71).—Consists of 1 or 2 liter Erlenmeyer flask into which is inserted close-fitting rubber stopper supported on stiff brass rod  $5/32$ – $6/32$ " in diam. and ca 3" longer than height of flask. (Rod of greater diam. is not desirable because of its greater displacement of liquid.) Rod is threaded at lower end and furnished with nuts and washers to hold it in place on stopper. Lower nut and washer must be countersunk in the rubber to prevent striking flask.

(b) *Büchner or Hirsch funnels for filtration with suction*.—Büchner or Hirsch funnels can be used interchangeably. Use Hirsch funnels with filter papers cupped up on sides to eliminate possibility of any of solids slipping under paper. (The No. 1A Coors Büchner funnel will accommodate the 7 cm filter paper. For filtration thru a Büchner or Hirsch funnel the use of suction and rapid-acting filter paper is assumed.)

Wire screen or bolting cloth placed between the perforated funnel plate and filter paper accelerates filtration. To facilitate microscopic observations, filter papers should be rapid acting to allow starch, etc., to pass thru; smooth; and ruled in oil-, alcohol- and water-proof lines. (Such a paper is the 7 cm ruled Shark Skin supplied by Schleicher & Schüll Co., New York.) (Grid consisting of metal ring ca 8 cm inside diam. and strung with fine parallel wires 7 mm apart can be placed over unruled papers; such a grid can be purchased from the Redman Scientific Company, San Francisco, California.)

(c) *Howard mold-counting slide*.—Glass slide of one-piece construction. In center of plate is flat plane ca 19 mm in diam. or 19 mm square, which is surrounded by moat and flanked by shoulders on each side 0.1 mm higher than the plane surface. Cover-glass is supported on shoulders and leaves depth of 0.1 mm between underside of cover-glass and the plane surface. The central plane, shoulders, and cover-glass have optically worked surfaces. To facilitate calibration of microscope, the newer slides are engraved with circle 1.382 mm in diam. or, in case of the square cell, have 2 fine engraved parallel lines 1.382 mm apart. An accessory micrometer disk for mold counting fits into the microscope eyepiece and is ruled into squares, each side of which is equal to  $\frac{1}{4}$  of diam. of eyepiece diaphragm opening. For mold counting, microscope must give an area of 1.5 sq mm (circle 1.382 mm in diam.) at magnification of 90–125.

(d) *Counting plate and cover for rot fragments*.—Plates are of glass 1.5–4.0 mm thick and covers are ca 1.5 mm thick. Dimensions of plates: 55 mm  $\times$  100 mm; rulings: crosswise, parallel lines 4.5 mm apart, with one 15 mm space at each end. One-half of square cover-slip ca 22 mm on a side and ca 0.25 mm thick is fastened at each end of counting plate by balsam to separate it from the cover-plate. Covers are 50 mm  $\times$  85 mm (Fig. 72).

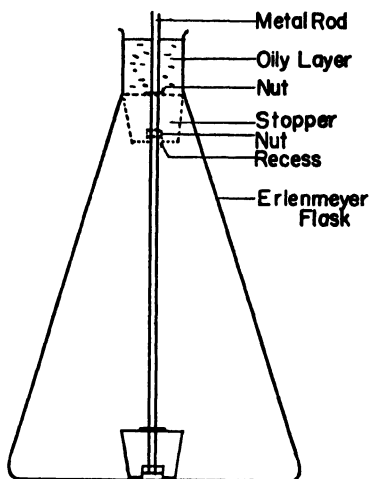


FIG. 71.—WILDMAN TRAP FLASK

\* Unless otherwise designated all methods in this chapter are tentative.

(e) *Blood counting cell*.—Preferably ruled in the Thoma or old Neubauer system. The so-called “improved” system of Neubauer may be used if depth of chamber is  $\frac{1}{16}$  mm and proper sized area is selected for counting. This cell is equipped with optically worked cover-glass.

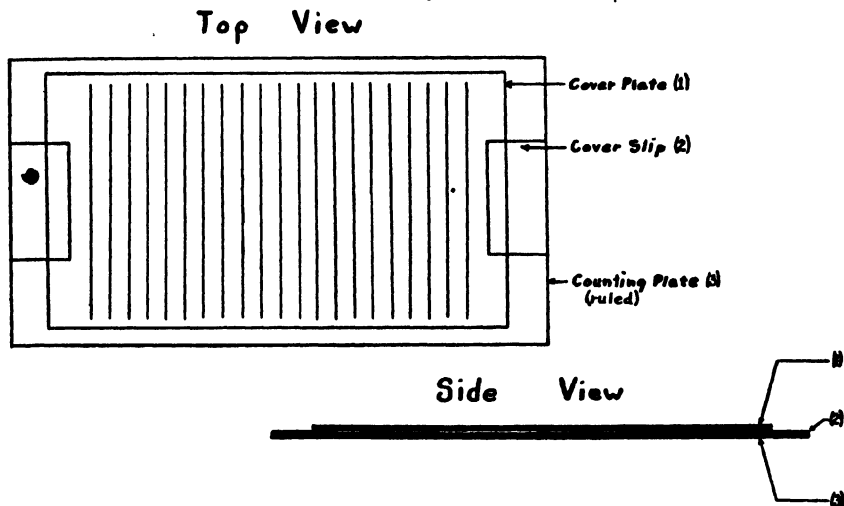


FIG. 72.—COUNTING PLATE

(f) *Petri dishes*.—Used to hold filter papers, bolting cloths, etc., for microscopic examinations; preferably should be of low-edge (10 mm high) type.

(g) *Coffer dam for Büchner funnel*.—Used to retain all filth particles on filter paper and to prevent them from slipping around edges. See Figure 73.

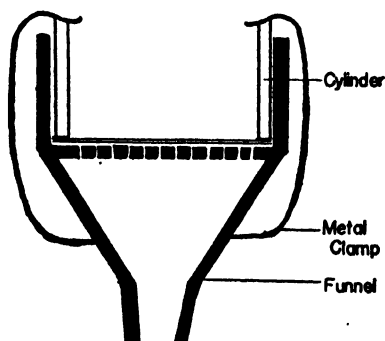


FIG. 73.—COFFER DAM

(h) *Water Filters*.—Where ample continuous supply of filtered  $H_2O$  is needed, a permanent arrangement should be installed. Two inexpensive “homemade” filters are described below. They may be constructed from materials available in most laboratories.

(1) Constructed from flared test tube held to bottom nonsplash portion of an aspirator by the screw cap or metal ring that normally holds wire screen on aspirator. The filter disk is supported on wire screen and when flange of test tube is tightened against disk a sufficiently tight seal is obtained.

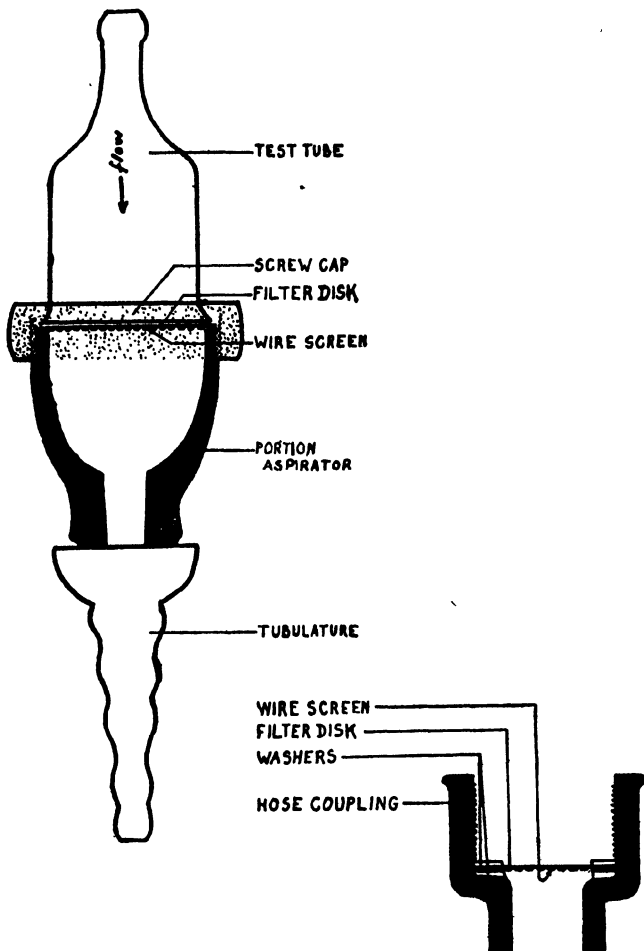


FIG. 74.—FILTERS

(2) Constructed from garden hose female coupling with wire screen and 2 filter disks placed between 2 thin washers so that when used on threaded faucet spout the assembly is sealed. Washers may be cut from rubber or leather, with appropriate sized cork borers, and the disks may be cut from Credicott sediment cloth, or filter paper disks may be used. For the wire disks a No. 60 sieve is preferred.

With either device (1) or (2) the disks may be removed at regular intervals for examination. The glass tube affords ready means of observing amount of sediment collecting on filter.

(i) *Sieves*.—As designated by U. S. numbers and defined in Circ. Letter LC 584, U. S. Dept. Commerce, Nat. Bur. Standards "Standard Specifications for Sieves," March 1, 1940.

(j) *Greenough-type binocular microscope*.—Illumination must be sufficiently intense and evenly distributed over field of view. At 20–25 $\times$  an area 12–14 mm in

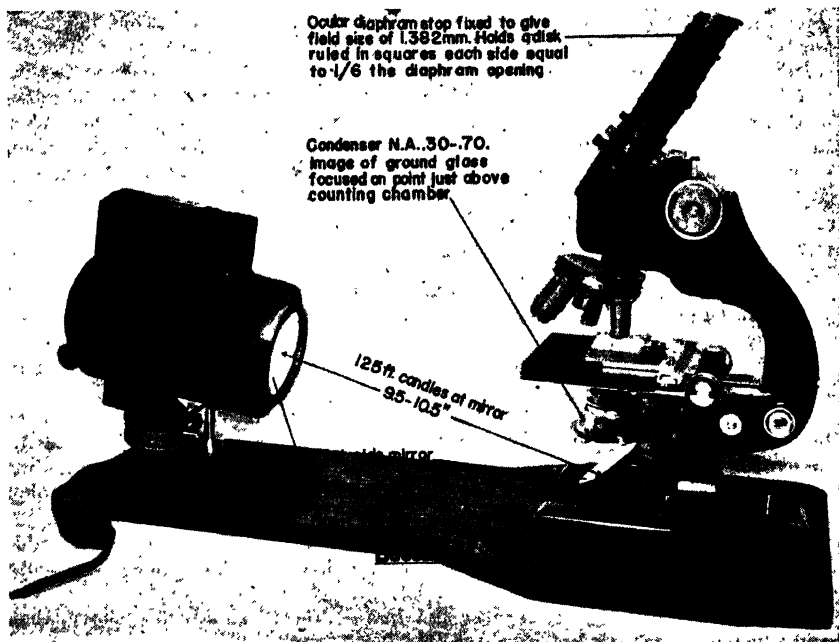


FIG. 75.—COMPOUND MICROSCOPE FOR MOLD COUNTING

diam. should be illuminated with ca 290 foot-candles. At 25–30 $\times$  the field of view will be smaller, but greater intensity of illumination will be needed. Where transmitted light is required light should pass thru object from below.

(k) *Compound microscope for mold counting*.—Provided with even illumination from light filtered thru blue or daylight ground glass. (Such illumination can be obtained with Bausch & Lomb Spherical Microscope Lamp or their Research Lamp, or with the Spencer Adjustable Laboratory Lamp No. 370 by focusing the lamp filament ca at infinity before inserting ground glass.) Place ground glass  $9\frac{1}{2}$ –10 $\frac{1}{2}$ " from microscope mirror pivot and so adjust with respect to lamp iris diaphragm, accessory resistances, etc., that 125 foot-candles of light are delivered to microscope mirror. Keep optical axis including lamp, mirror, iris, condenser, and objective in alignment. To accurately and individually center each element with all others insert top element of condenser. Have ground-glass illuminant at right angles to optical axis.

The light-mirror-objective relationship can usually be checked by opening condenser iris, removing eyepiece, and centering light image while looking into empty eyepiece tube. Iris and condenser can usually be centered by having eyepiece in place and focusing objective on smallest iris opening. Then center opening in field of view. Similarly, check lamp and mirror alignment by focusing on lamp iris or on mark on ground glass and centering it by moving mirror.

Fasten lamp and microscope securely to baseboard so that they are used and maintained as a unit (e.g., B. & L. baseboard 31-50-54). Have mirror pivot tight-fitting so that it is not too easily moved, and hold microscope in place by screws or cleats. (Simple and inexpensive arrangement for holding lamp and microscope in proper position and alignment is shown in Fig. 75. The baseboard is wood.)

Use flat side of mirror. Also use condenser with upper element removed so that maximum numerical aperture is .30-.70. When in use place condenser at such distance from slide that image of ground glass is focused at same plane as objective. In other words, focus objective upon mold-counting field and at same time upon image of ground glass as projected by condenser. Then move condenser up *slightly* or *just enough* to remove from field the "sandy" background of light. Regulate light intensity with the iris diaphragm.

#### 42.2

#### REAGENTS

Unless otherwise specified all  $H_2O$  is distilled or tap  $H_2O$  that has been filtered and boiled. Make all alcohol dilutions by volume, and in filth work filter all reagents whenever practicable.

(a) *Petroleum benzine and ether.*—Use interchangeably. (Because of cost, petroleum benzine may be preferred.)

(b) *Stabilizer solns (pectin, carob bean, algin).*—Add required quantity of stabilizer directly to  $H_2O$  while in agitation in a blender. Treat soln with vacuum or heat to remove air bubbles. As a preservative add 2 ml of  $HCHO$  soln/100 ml of stabilizer mixture. (When blender is not available, dry stabilizer may be mixed with alcohol to facilitate its incorporation with the  $H_2O$ .)

(1) *Carob bean soln.*—Proceed as directed above, then boil and cool before adding  $HCHO$  in order to cause separation of insoluble vegetable elements, and use clear supernatant soln.

(2) *Algin soln for rot fragment determination.*—Proceed as directed above, then adjust final mixture with  $NaOH$  soln to pH 7.0-7.5.

(c) *Pancreatin soln.*—For each half pound of sample to be treated, mix 5 g of pancreatin with ca 50 ml of  $H_2O$  and allow to stand 30 min. Centrifuge and pass supernatant liquid thru loose mat of cotton without suction, and then filter thru rapid filter paper in Hirsch funnel.

(d) *Arsenophosphotungstic acid.*—Dissolve 25 g of  $Na_2WO_4 \cdot 2H_2O$  in 150 ml of  $H_2O$  and add 12.5 g of  $As_2O_5$ , 6.2 ml of  $H_3PO_4$ , and 5 ml of  $HCl$ . Boil mixture 20 min., cool, and dilute to 250 ml with  $H_2O$ .

(e) *Sodium cyanide soln.*—Dissolve 5 g of  $NaCN$  in  $H_2O$ , add 0.2 ml of  $NH_4OH$ , and dilute to 100 ml.

#### 42.3

#### GENERAL INFORMATION AND PRECAUTIONS

Do not use violent agitation as provided by malted milk stirrers, rapid mechanical beaters, grinders, etc., unless specified. Do not use strong alkali. Boiling in 1%  $NaOH$  soln dissolves rodent hairs, and boiling in 1%  $Na_3PO_4$  soln 5 min. swells and distorts them. Foods, if present, afford some protection. Also avoid use of  $HNO_3$  and  $H_2SO_4$  at greater concn than 5%; 5%  $HCl$  is much safer. Rodent hairs will hold up 15-40 min. in boiling 5%  $HCl$ , and in 5%  $H_3PO_4$  and other relatively weak acids.

Insect parts are much more resistant to acids or alkalis than are hairs. They are softened and lose their color in alkali, but are not dissolved in the most concentrated aqueous KOH solns. Extended boiling in strong acids may carbonize them.

Insects, insect fragments, and rodent hairs are often lighter than the food in which they are found, and may sometimes be floated out in heavier-than-water liquids while the plant tissue settles out. Usually, however, they are extracted by a different procedure. With the exception of fly larvae or maggots, insects and insect fragments can be wet with oils, mixed into an aqueous mixture of a food, and so floated to surface with the oil. In practice, several factors prevent complete separation. It is difficult to wet all insect material without creating frothy emulsion of the plant material that will obscure subsequent examination. Fragments may become trapped in, or attached to, a mass of plant material settling out. Droplets of oil adhering to sides of trap flask often keep insects from rising. To obviate some of these effects, work the oil or gasoline thoroly into the  $H_2O$ -food mixture, but use no "whipping" and avoid inclusion of air. Provide intermittent agitation while separation is taking place. Some products cannot be extracted in  $H_2O$  because too much of food might rise with the "light filth." To reduce floury emulsions, the extractions can be made in saturated NaCl soln. Sometimes capryl alcohol or alcohol can be used to break an emulsion. In general, when much bran or chaff is present it will float up with the oil when  $H_2O$  or saturated NaCl soln is used, and it is advisable to do the extracting in an aqueous-alcohol soln. (For some cereals an aqueous isopropyl alcohol soln may be used.) The alcohol not only soaks into bran, but it is also less dense, so less plant tissue floats. Material separated in one Wildman trap may be transferred to another trap and re-washed to remove some of plant material, but in many instances any additional operation causes loss of filth material and is to be avoided.

#### 42.4

#### SPECIAL TECHNIQS

(a) *Operation of Wildman trap flask.*—Place the liquid-food mixture in the flask, add gasoline, and stir it in. In some cases, care must be taken that frothy emulsion is not created and too much of food material floated to surface. After the gasoline has been mixed in, "trap off" by adding sufficient liquid to bring floating oily layer up into neck of flask so that when stopper is raised floating layer and ca 1 cm of liquid below interface are entrapped and decanted. Spin stopper to remove adhering food before raising it.

When decanting, hold stopper in place, and rinse out material adhering to rod and neck of flask with alcohol and then with  $H_2O$ . Filth that adheres to walls of containers owing to surface attraction must be rinsed off and recovered. Unless otherwise stated, allow mixtures to stand at least 30 min. while separation occurs, and stir bottom layer every 3–6 min. to release filth fragments caught there and on sides of flask.

The trapped-off mixture is usually filtered thru rapid filter paper into Hirsch or Büchner funnel. Sides of filter must be washed down.

When the extraction yields a large amount of filth, second trapping off by following procedure is mandatory: Add to the trap flask 10–20 ml of oil, or gasoline, and stir in vigorously; add ca 10 ml of  $H_2O$  or of liquid in which flotation is being made; allow to stand 10–20 min. with intermittent stirring of bottom layer, trap off, and filter. If filter tends to clog, moisten paper with isopropyl alcohol or 40% alcohol before adding mixture. Filtering mineral oil may be washed thru with  $CHCl_3$ .

(b) *Preparation of filter paper for examination.*—With the Wildman flask some of food material is often trapped off with the filth particles, but by proper clearing these may be made to stand out by contrast on the white background of the filter paper by the following procedures:



(1) Wash filter paper, while it is still in Büchner funnel, with alcohol or acetone or both, draw air thru it until paper is dry, then transfer to Petri dish to which ca 5 ml of heavy mineral oil has been added. The oil will clear the paper and material on it. When cleared, smooth upper surface of filter paper by adding mineral oil, and then examine.

(2) Simply moisten filter with  $H_2O$  or 70% alcohol. (This method does not clear completely, but it leaves rodent pellets and other filth soft and pliable.)

(3) Procedure (1) often gives paper that does not lie flat in Petri dish, and that clears unevenly. Following method is slower but gives well-cleared, flat paper: Rinse material into Büchner funnel with  $H_2O$  or 20–40% alcohol, draw air thru until paper is no longer dripping wet, and then transfer paper to Petri dish containing ca 5 ml of heavy mineral oil. Adjust paper until layer of oil lies under entire surface and holds paper to dish. The  $H_2O$ -alcohol will evaporate gradually and oil will work up, clearing and hardening paper in flat condition.

(4) When considerable amount of extraneous matter is present, clove oil may be substituted in (1) or (3). This oil has relatively high refractive index and clears more completely than does mineral oil.

(5) Instead of using (4), wet or dry paper may be cleared and starchy material completely gelatinized by heating with chloral hydrate in  $H_2O$  or glycerin soln. The chloral hydrate is extremely noxious to use, but it can be washed out of the filth after the clearing and before the microscopic examination. (Chloral hydrate will dissolve most filter papers.)

(c) *Microscopic examination of filter papers.*—Made with the binocular Greenough-type microscope at 20–30 diam. Remove doubtful fragments to slide, mount in mineral oil or glycerin, etc., and examine under compound microscope. *Thoro understanding of appearance of authentic material is assumed.*

## CANNED CITRUS JUICES

### 42.5

#### MOLD COUNT

Pour contents of can into beaker and thoroly mix by pouring back and forth between beaker and can at least 12 times. After mixing, transfer 50 ml of juice to graduated 50 ml conical-bottomed centrifuge tube. Centrifuge 10 min. at 2200 r.p.m., using International S.B. type, size 1 centrifuge, with No. 240 8-place head. (Distance from center of centrifuge head to center of cups (at rest) is  $5\frac{1}{2}$ ".) If this type of centrifuge is not available, use sufficient speed with any available centrifuge to obtain equivalent centrifugal force, which may be computed by following formula:  $N_2r_2 = N_1r_1$ , where  $N$  = revolutions / min. and  $r$  = radius of centrifuge arm. Check speed with a tachometer, since the rheostat does not necessarily indicate speed in r.p.m. Let centrifuge gradually come to complete stop before removing tubes. Remove tube and decant supernatant liquid without disturbing sediment. Add  $H_2O$  to tube to bring level to 10 ml mark and then add 5 ml of 3% pectin soln, 42.2(b). Thoroly mix sediment,  $H_2O$ , and pectin soln and pour into small beaker. Mix by pouring back and forth between beaker and tube at least 6 times. Stir mixture thoroly in beaker and proceed as directed in 42.57.

### 42.6

#### FLY EGGS AND MAGGOTS

Pour 250 ml of thoroly mixed sample directly into suction flask thru Büchner funnel fitted with piece of 10XX bolting cloth (wire mesh screen under bolting cloth facilitates action). Pour juice slowly to avoid accumulation of excess pulp tissues on filter cloth (2 or 3 cloths may be necessary).

## 42.7

## INSECT FRAGMENTS AND RODENT CONTAMINATION

Pour 250 ml of juice into 2 liter Wildman trap flask. Add 15 ml of castor oil, and fill flask with hot  $H_2O$  (ca  $50^\circ$ ), stirring vigorously. Add enough hot  $H_2O$  to bring oil layer into neck of flask. Let stand 30 min. Trap off and pour into beaker, and rinse out neck of flask with alcohol, adding this to beaker. Filter, rinsing out beaker with alcohol.

## TEA, LEAFY CRUDE DRUGS, AND CONDIMENTS

## 42.8

## RODENT AND INSECT EXCRETA

Weigh 25 g of sample and transfer to 1-quart conical glass percolator (Fig. 76) with cork stopper A in place. Add ca 300 ml of  $CHCl_3$ , mix thoroly, and allow to settle at least 30 min., stirring several times the layer that rises to top. With plunger work down any heavy filth that has collected on shoulder above tube outlet. Fix cork B into position, trapping heavy filth between A and B. Withdraw cork A and catch sediment and  $CHCl_3$  in small beaker. Rinse percolator tube by slightly raising cork B. Wash contents of beaker with  $CHCl_3$  onto 7 cm ruled filter paper in Büchner or Hirsch funnel. Transfer paper to Petri dish and examine microscopically.

42.9 INSECTS, INSECT FRAGMENTS,  
AND HAIRS

Transfer sample and  $CHCl_3$  remaining in percolator to Büchner funnel. Filter out  $CHCl_3$ , and air dry until no  $CHCl_3$  odor remains. Transfer material to 2 liter Wildman trap flask. Add ca 600 ml of  $H_2O$ , bring to boil, and simmer 10–15 min., stirring material down into the liquor several times. Wash down sides and rod with  $H_2O$  and cool to room temp. Stir in 40 ml of light mineral oil, fill flask with  $H_2O$ , let stand 30 min., trap off, and filter. Make second extraction onto same paper by stirring 15–20 ml of mineral oil into material, trapping off, allowing to settle 10–15 min., and filtering. Remove filter to Petri dish and examine microscopically.

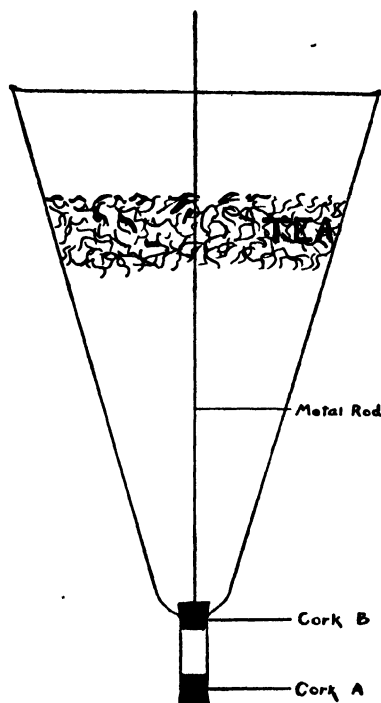


FIG. 76.—CONICAL GLASS PERCOLATOR

## COCOA, IMITATION COCOA, AND COCOA SUBSTITUTES

## 42.10

## FILTH

Weigh 25 g sample in 250 ml beaker, add 150 ml of  $CHCl_3$ , and boil 15 min., stirring occasionally. Wash down sides of beaker with  $CHCl_3$ . Allow mixture to cool and settle 15 min., with occasional stirring of top layer. Decant  $CHCl_3$  and floating tissue onto smooth 7 cm filter paper in Büchner funnel, taking care not to disturb heavy residue in bottom of beaker. Repeat with small quantities of  $CHCl_3$  from wash bot-

tle until practically no plant tissue remains with residue on bottom of beaker. Transfer residue from beaker to ashless filter paper and examine for filth. If residue is appreciable, ignite filter and determine weight of sand, soil, etc.

Draw air thru the material in Büchner funnel until solvent has evaporated. Transfer dried material to 1 liter Wildman trap flask and add 150 ml of hot  $H_2O$ . Boil 15 min., and, if necessary to prevent foaming, direct stream of air into mouth of flask. Cool mixture below  $20^\circ$ , add 25 ml of gasoline, and mix vigorously. Wash down inside of flask with  $H_2O$  and allow mixture to stand 5 min. Fill flask with  $H_2O$  and allow to stand 30 min. with occasional stirring. Trap off, filter, and examine.

## DAIRY PRODUCTS

### 42.11

#### MANURE FRAGMENTS

Filter 500 ml of fluid milk thru conventional type sediment pad. For other dairy products select method using  $Na_2C_2O_4$ , Na citrate,  $H_3PO_4$ , or  $H_2O$  for isolation of insoluble filth elements. Transfer ca 100 vegetable fragments (max. length 2 mm) selected at random to 3–4 ml of  $H_2O$  in small casserole, using tweezers; then transfer to No. 1, 30 ml Gooch crucible fitted with disk of hard-surfaced filter paper, using a stream of hot  $H_2O$ . Wash thoroly with stream of hot  $H_2O$  (ca  $90^\circ$ ), then with hot alcohol, and finally rewash with hot  $H_2O$ . Transfer fragments back to casserole, containing a drop of  $H_2O$ , with tweezers. (For this purpose the small disk of filter paper may be placed on microscope slide and moistened with drop of  $H_2O$  to facilitate removal of particles back to casserole.) Add, dropwise, 10 drops of 1% aqueous Fast Green FCF soln. Push floating fragments beneath surface of staining soln to insure wetting. From time of addition of dye soln, allow mixture to stand 15 min. (within  $\frac{1}{2}$  min.). Wash particles free of staining soln by filtering onto Gooch crucible, using fine stream of cool  $H_2O$  from wash bottle. Transfer particles back to casserole, using a *very fine* stream of  $H_2O$  from wash bottle to wash particles from filter paper (3–5 ml of  $H_2O$  is sufficient). Bring mixture to boil, but do not permit particles to adhere to sides of casserole as charring may result, and boil 15 seconds. Filter *immediately* thru Gooch crucible, using stream of  $H_2O$  from wash bottle to rinse all fragments into crucible, and transfer to casserole, containing a drop of  $H_2O$ , using tweezers. Repeat staining and boiling procedure and filter immediately thru 7 cm filter paper (cross-sectioned in 6 mm squares) in Büchner funnel. Allow paper to dry and place in Petri dish containing 1–2 ml of mineral oil. Segregate manure fragments from all others, using low-power microscope (ca  $10\times$ ). Count and report number and percentage of manure particles on following basis: Manure fragments of plant origin after staining have characteristic dirty worn appearance and brownish translucent color, and may or may not be slightly green. Presence of irregular, patternless, amorphous specks on surface of plant fragments is positive additional evidence of manure, and majority of plant fragments from manure have them; their absence, however, does not preclude the fragments being classified as manure. Undigested plant fragments have typical green color and/or other outstanding characteristics when compared to digested plant tissue.

## CHEESE PRODUCTS

### FILTH

### 42.12

#### Quantitative separation

Cut 225 g of cheese into cubes 25 mm across, or smaller, and add reagent selected in appropriate sized beaker. Provide means of heating or maintaining proper temp. Proceed as directed below, or as in 42.13(a), (b), or (c) after substituting butter

mixer, 22.109, for Waring Blendor or malted milk mixer and a filter paper for the sediment pad.

(a) *Sodium citrate*.—Heat 400 ml of 15% Na citrate soln in 1500 ml beaker to 65°, add cheese and stir. Maintain temp. close to 60° (not above 62°). After stirring 5–15 min., add 200 ml of H<sub>2</sub>O and 200 ml of the Na citrate soln (both heated to 60°). Continue stirring 5–15 min. longer and filter thru paper, keeping stream of hot H<sub>2</sub>O on paper to facilitate filtration.

(b) *Phosphoric acid*.—To 1500 ml of hot H<sub>2</sub>O in 2 liter beaker add 25 ml of H<sub>3</sub>PO<sub>4</sub> (75% technical grade). Heat to 95–100°, insert stirrer paddles, and add cheese. Stir ca 5 min. and filter thru paper.

(c) *Hydrochloric acid-sodium citrate-ammonia*.—To 350 ml of hot H<sub>2</sub>O in 1500 ml beaker, add cheese, and stir 1½–2 min., or until cheese is dispersed. While mixing, adjust to pH of 2 or lower with HCl. Cool to 50° or lower and add 70 ml of 40% Na citrate soln. Add slowly and with rapid stirring sufficient NH<sub>4</sub>OH soln (1+9) to adjust to pH 6–7. Stir 10 min. and filter thru paper.

#### 42.13

#### *Qualitative separation*

Proceed as directed in 42.12 or below, but filter thru sediment pad having filtering area 1" in diam. Use 225 g sample.

(a) *Hydrochloric acid*.—Add cubed cheese and 300 ml of cold H<sub>2</sub>O to Waring Blendor and mix until smooth consistency is obtained. Rinse into beaker and dilute to 1.5–2 liters with H<sub>2</sub>O. Add with stirring 5 ml of HCl for each 100 ml of liquid and heat to boiling. Boil ca 2 min., and, if clumps are still present, mix liquid with malted milk mixer. Filter thru sediment pad.

(b) *Pancreatin*.—Mix cheese and 300 ml of cold H<sub>2</sub>O in Waring Blendor or with malted milk mixer. Adjust mixture to pH of 8 and add 300 ml of 10% pancreatin soln previously prepared by centrifuging and filtering thru cotton and then paper. Keep at 35–45° ca 5 min., readjust to pH 8, and continue digestion 1 hour. Acidify with H<sub>3</sub>PO<sub>4</sub> to pH 2, and boil vigorously 15 min. Add 500 ml of alcohol, heat to boiling, and filter thru sediment pad.

(c) *Sodium citrate*.—Place 100 ml of 40% Na citrate soln in Waring Blendor, add 150 ml of cold H<sub>2</sub>O, start mixer, and add cubed cheese. Mix to smooth consistency (15 seconds–2 min.). Transfer to liter beaker, add H<sub>2</sub>O to make total of 800 ml, and heat with stirring to 60°. Maintain at this temp. 15 min. with frequent stirring. Filter thru sediment pad.

(d) *Hydrochloric acid-sodium hydroxide method for cream cheese with gum*.—Place 300 ml of warm H<sub>2</sub>O (60°) and the cubed cheese in Waring Blendor and mix ca 15 seconds. Transfer to 1500 ml beaker and bring to boil with stirring. Add 50 ml of HCl (1+1) slowly with rapid stirring. Boil 5 min., cool to ca 60°, add 1 ml of 1% phenolphthalein soln, and neutralize with 20% NaOH soln. Adjust to pH of ca 11.5 (preferably not higher) with more alkali. Heat to 60° and maintain at this temp. 10 min. Dilute with equal volume of hot H<sub>2</sub>O (60°) and filter thru pad.

(e) *Hydrochloric acid-sodium citrate-ammonia*.—Proceed as directed under 42.12 (c), except to use Waring Blendor for the mixing prior to cooling to 50°. At this stage transfer mixture to beaker and continue as directed under 42.12(c) but filter thru sediment pad.

#### 42.14

#### MITES

In examination of numerous samples of cheese for mites it has been observed that infestation is confined to outer portions.

Remove wrapper from brick cheese and cut section, 3–4 mm deep, parallel to sur-

face of each face of brick. Break sections into pieces ca 15 mm across and drop into liter Wildman trap flask, 42.1(a), containing 200 ml of 60% alcohol and 50 ml of gasoline. Stir contents thoroly and continue to add more 60% alcohol until gasoline interface rises into neck of flask. Let stand at least 5 min., trap off gasoline layer, and filter. Continue to add 60% alcohol until liquid again rises into neck of flask, mixing thoroly. Let stand 5 min. more, then trap off and filter. Examine filter paper for mites.

### DRIED MILK

#### FILTH

#### 42.15

##### *Filtration on paper*

Mix 250 g of powder with 700 ml of H<sub>2</sub>O 1–2 min. with butter stirrer, 22.109. Rinse stirrer, and add 150 ml of 10% pancreatin soln previously prepared by centrifuging and filtering thru cotton and then paper. Mix thoroly and adjust with NH<sub>4</sub>OH (1+9) to ca pH 8, using indicator paper. Heat to 35–45° 30 min. Adjust to pH 2 with HCl and boil 15 min. Add slowly equal volume of alcohol and heat to boiling on electric hot plate. Filter while hot thru paper. Add boiling H<sub>2</sub>O until all soluble material has been filtered. Examine paper microscopically.

#### 42.16

##### *Filtration on sediment pad*

To 300 ml of cold H<sub>2</sub>O in Waring Blendor gradually add 100 g of the milk powder. Avoid prolonged mixing because of excessive foam formation. (Total mixing time need not exceed 15–20 seconds.) Transfer mixture to liter beaker, rinsing Blendor with ca 150 ml of H<sub>2</sub>O. Stir into milk mixture 100 ml of filtered 40% Na citrate soln. Heat to 60° while stirring and maintain at this temp. with frequent stirring 10–15 min., or until milk powder is dissolved and soln appears translucent. Filter hot soln on milk sediment pad with cloth covering. (Any foam on soln will pass thru pad without difficulty.) Use a little hot H<sub>2</sub>O from wash bottle to assist in transferring foam and overcoming any tendency of filtration to slow down.

### CREAM

#### 42.17

#### FILTH

(a) *For most creams.*—Heat ca 1 liter of H<sub>2</sub>O in Al pan (ca 2 qt.) to 65–70°. Add 500 ml (ca 1 pint) of cream and stir with thermometer. (Ordinarily temp. will drop to 52–60°). If temp. falls below 53°, heat mixture until it comes up to 53–60°. Do not heat over 60°. Place pan under malted milk mixer and stir. In case of high acid cream, add 10% NaOH soln in portions of 1–2 ml until mixture turns red litmus paper a faint blue color. Add 30 ml of 40% Na hexa-metaphosphate soln and heat to 55°. (For most creams the NaOH treatment is unnecessary.) After stirring a moment, filter thru sediment pad. Wash with hot H<sub>2</sub>O. Avoid excessive stirring. If pad clogs, transfer mixture from funnel back to pan, add 30 ml more of the Na hexa-metaphosphate soln, stir 1 min., and filter again thru fresh pad.

(b) *For cream that cannot be filtered.*—Heat ca 1 liter of H<sub>2</sub>O in 2 liter beaker to 65–70°. Add 500 ml (ca 1 pint) of cream and stir with thermometer. (Ordinarily temp. will drop to 52–60°.) If temp. falls below 53°, heat again to 53–60°. Place beaker under butter mixer, 22.109, and while stirring add 10% NaOH soln in 5 ml portions until mixture turns litmus paper decided blue. Add 30 ml of 40% Na hexa-metaphosphate soln (a) and continue stirring 5 min. Pour contents of beaker into 2 liter separator, washing beaker with hot H<sub>2</sub>O from wash bottle. Allow suspension to remain in separator 30 min., swirling separator at 5 min. intervals to accelerate

separation of sediment. Then draw from bottom of separator 500 ml of liquid containing the sediment and filter thru sediment pad. Drain remainder of material in separator into 2 liter Wildman trap, 42.1(a), and wash separator with hot H<sub>2</sub>O from wash bottle. Add to trap 40 ml of gasoline and mix in with gentle rotary motion. (Too vigorous stirring results in formation of emulsion that will clog sediment pad in following step.) Fill trap with H<sub>2</sub>O. After 30 min. trap off the gasoline layer thru same sediment pad used previously. Pour remainder of material in Wildman trap thru bolting cloth (110-mesh). Where it is not possible to filter the 500 ml drawn from bottom of separator thru sediment pad wash material on pad back into beaker containing balance of the 500 ml portion and allow to stand 30 min. Decant contents of beaker, with exception of 50–100 ml, back into separator. Then pour the 50–100 ml portion thru clean sediment pad and treat contents of separator as directed for gasoline flotation procedure, 42.4.

## EVAPORATED AND CONDENSED MILK

### FILTH

#### 42.18

#### *Filtration on paper*

Place 225 g of milk in 1500 ml beaker and add 500 ml of boiling 3% Na<sub>2</sub>C<sub>2</sub>O<sub>4</sub> soln while stirring. Stir ca 1 min. and filter thru filter paper. Wash filter with warm H<sub>2</sub>O. Examine paper microscopically.

#### 42.19

#### *Filtration on sediment pad*

- (a) Proceed as directed under 42.18, but filter on sediment pad; or
- (b) Mix 225 g of product into 1500–2000 ml of boiling H<sub>2</sub>O and filter thru pad.

### BUTTER (2)

#### 42.20

#### MOLD—OFFICIAL

Make careful examination of surface of sample and note any visible mold growth. To remove possibility of contamination by surface mold, scrape off and discard  $\frac{1}{4}$ " of surface. Weigh out 1 g of butter obtained from exposed surface in 50 ml beaker. Add 7 g of hot (50–60°) gum soln prepared by making either 0.75% soln of carob bean gum, 42.2(b)(1), or 3% soln of pectin or other gum, 42.2(b), of similar viscosity. Stir until mixture is uniform and fat globules are 0.1–0.2 mm in diam. Mount portion of the mixture on Howard cell, 42.1(c), and estimate mold as directed in 42.57. Consider fields positive when single filament or combined length of two longest filaments exceeds  $\frac{1}{2}$  of diam. of field.

(a) *Alternative procedure (staining)*.—Add 1 or 2 drops of 5% crystal violet soln to the gum-butter mixture after butter is melted. Mix preparation thoroly and prepare slide as directed above.

#### 42.21

### FILTH

(a) *Filtration on paper*.—Heat 225 g of butter in liter beaker with 200 ml of 2% HCl, with continuous stirring during latter part of heating, until mixture is boiling. Use butter mixer for stirring if many samples are to be run. Filter at once thru 7 cm rapid-acting paper supported on 40-mesh brass screen in Büchner or Hirsch funnel. Do not allow mixture to accumulate on paper. When filtration is impeded, add hot H<sub>2</sub>O until paper is cleared, then add small amounts of the butter mixture alternately with hot H<sub>2</sub>O.

**NOTE:** Samples that foam strongly when heated to boiling usually filter slowly at first. Such samples are frequently, but not invariably, helped by addition of 10 ml of alcohol, with mixing, just prior to filtering. With some samples that are difficult to filter, continuous heating and stirring for 5–10 min. will be of assistance.

(b) *Filtration on sediment pad.*—Add 1500 ml of hot  $H_2O$  and 25 ml of  $H_3PO_4$  to 2 liter beaker and heat to 95–100° on electric heater. Insert paddles of mechanical stirrer described in 22.109 and add 450 g of butter in small portions. Stir 2–3 min. and filter thru a sediment pad so arranged that filtering area 1" in diam. is provided. Use 225 g of sample if 450 g will not filter. (This method may be used for quantitative filth determination if filter paper is used in place of sediment pad.)

## SHELLED NUTS

### 42.22

#### FILTH

(a) *Heavy filth.*—Thoroughly mix each subdivision of sample and weigh 100 g portions into 400 ml beakers. Add ca 200 ml of  $CHCl_3$  and allow to settle 10–15 min. Decant  $CHCl_3$  and nut kernels. Repeat if necessary to remove all nut particles. Transfer residue, if any, to ashless filter paper and examine for heavy filth. Ignite, and weigh to determine sand and dirt.

(b) *Light filth.*—Weigh 100 g into suitable beaker, cover with hot (60–70°) 1% Na oleate soln, and bring to boil with intermittent stirring. (CAUTION: Soln has tendency to foam.) Decant the Na oleate soln into 2 liter Wildman trap flask, 42.1(a), thru sieve of suitable mesh (as large as possible without allowing nut meats to fall thru). Place thin layer of nut meats on screen and wash with fine, strong stream of  $H_2O$ , successively removing layer and adding more nuts until entire sample has been rinsed. Discard nut meats. Catch all washings in the trap flask. Cool washings and Na oleate soln below 20°. Add 35 ml of gasoline and mix thoroughly. Fill flask with  $H_2O$  and allow to stand 30 min., stirring occasionally. Trap off, filter, and examine microscopically.

## PEANUT BUTTER

#### FILTH

### 42.23

#### PREPARATION OF SAMPLE

Examine individually at least 3, and preferably 6, jars. If the jars contain less than 1 lb. each, make 3–6 composites of at least 2 jars each so that composite samples will be ca 1 lb. Remove contents of each jar and mix thoroughly, preferably in evaporating dishes of convenient size, using heavy table fork or spatula. The peanut butter after warming may also be mixed in the jar, using electric mixer described in 22.109 (should be equipped with stiff paddles). If large number of jars are to be examined, make composite samples by thoroughly mixing the contents of 3–6 jars of equal size.

### 42.24

#### WATER-INSOLUBLE INORGANIC RESIDUE ("WIIR") AND EXCRETA

Weigh 100 g portions of each sample into 250 ml beakers (preferably hooked-lip type), add ca 10 ml of petroleum benzine, and mix thoroughly. Continue to add the petroleum benzine, and mix thoroughly, until ca 150 ml has been added. Cover, allow mixture to settle 10 min., and decant ca  $\frac{3}{4}$  of petroleum benzine layer, being careful not to lose any of coarse peanut tissue. Pour all decanted petroleum benzine at once thru 100-mesh bolting cloth and examine cloth for filth that may have floated in the petroleum benzine. Add ca 100 ml of petroleum benzine to residue and mix. Allow to settle as before and decant. Add ca 75 ml of the petroleum benzine, stir, wash down sides of beaker with stream of petroleum benzine from wash bottle,

allow to settle 5 min., and decant. Evaporate balance of petroleum benzine from residue, using gentle heat if convenient. If sample is not to be examined for light filth, omit petroleum benzine treatment. Add 150 ml of  $\text{CHCl}_3$  to residue in beaker and mix thoroly; cover beaker, and allow to settle 20 min. Stir top layer several times during this period. Decant  $\text{CHCl}_3$  and floating peanut tissue onto smooth filter paper in Büchner funnel, being careful not to disturb heavy residue in bottom of beaker. Save all decanted peanut tissue for later determination of light filth. Repeat extraction with small quantities of  $\text{CHCl}_3$ , rinsing all particles from sides of beaker. At this point watch for fragments of rodent excreta pellets on top of  $\text{NaCl}$  in bottom of beaker; do not decant them. (If sample contains considerable peanut skin it may be necessary to use a mixture of  $\text{CHCl}_3$  and  $\text{CCl}_4$  to float these particles away from heavy residue of  $\text{NaCl}$ , sand, etc.) Dry residue in air.

Add 50 ml of  $\text{HCl}$  (1+35) to residue in beaker, then add 90 ml of boiling  $\text{H}_2\text{O}$  and allow to stand 30 min. with occasional stirring, which should dissolve any phosphate, carbonate, or anhydrite ( $\text{CaSO}_4$ ) included with the  $\text{NaCl}$ . Decant liquid thru ashless filter in 60° glass funnel and finally transfer residue with hot  $\text{H}_2\text{O}$ . Test filtrate for  $\text{SO}_4$  by adding 5 ml of saturated  $\text{BaCl}_2$  soln. Wash residue on filter several times with hot  $\text{H}_2\text{O}$ . If above test for  $\text{SO}_4$  in filtrate was positive, test residue on filter by placing clean beaker or test tube under funnel and treating residue with 25 ml of  $\text{HCl}$  (1+35), adding a little at a time. Test filtrate with 20 drops of saturated  $\text{BaCl}_2$  soln (a fine white precipitate of  $\text{BaSO}_4$  indicates presence of anhydrite in residue on filter; allow 5 min. for precipitate to appear). Wash residue on filter with hot  $\text{H}_2\text{O}$  until all  $\text{HCl}$  is removed.

Remove filter paper. Using magnification of 20–40 diam., examine residue microscopically for rodent excreta pellet fragments (identified by presence of rodent hair fragments in the mass), insect excreta pellets, and other filth. Ignite filter paper in a tared crucible over medium Bunsen flame or in muffle furnace at ca 500°. Weigh crucible and contents to nearest 0.5 mg. If the “WIIR” is excessive and above test indicates that all the  $\text{CaSO}_4$  has not been removed, make a quantitative determination of either the  $\text{Ca}$  or the  $\text{SO}_4$  in the “WIIR” in the crucible, as directed in 37.113 and 37.114. Calculate this weight to  $\text{CaSO}_4$  and correct weight of the “WIIR”.

#### 42.25

#### LIGHT FILTH

Draw air thru Büchner funnel containing the peanut tissue, 42.24, until all  $\text{CHCl}_3$  is removed, or transfer peanut tissue to sheet of smooth paper and air dry. Transfer dry peanut tissue to 2 liter Wildman trap flask, 42.1(a). Add 200 ml of alcohol and boil 20 min. on hot plate or steam bath, stirring frequently. Add 200 ml of hot  $\text{H}_2\text{O}$  (not above 70°) and simmer 15 min. Wash down sides of flask. Cool mixture below 20°, add 35 ml of gasoline, and mix thoroly. Allow to stand 5 min. before filling flask with  $\text{H}_2\text{O}$  and then allow mixture to stand 30 min. Trap off, filter, and examine. If too much peanut tissue comes up under gasoline layer, pour material directly into liter Wildman trap flask and rewash with  $\text{H}_2\text{O}$  or 40–50% alcohol. Allow 20 min. for mixture to settle, trap off, and filter as before. Add ca 20 ml of gasoline to original flask and stir to draw gasoline down into mixture. Add sufficient  $\text{H}_2\text{O}$  to fill flask and allow to stand 20 min. Trap off, filter, and examine. If second extraction yields relatively large amount of filth, make third extraction after first decanting most of liquid from flask and adding 20 ml of gasoline to peanut tissue. Examine the filter papers. Pour mixture remaining in flask thru No. 40 sieve, wash material on sieve thoroly, and transfer to one or more Petri dishes. Add sufficient alcohol to cover material and examine microscopically, using magnification of 20–40 diam., for moldy and decomposed peanut fragments, rodent excreta and other filth.



**BAKED PRODUCTS, PREPARED CEREALS****42.26****PREPARATION OF SAMPLE**

Defat pie crusts, cakes, and cookies that are rich in shortening, by soaking in petroleum benzine and decanting off the petroleum benzine and fat, being careful not to carry off any hairs or insects. Air dry until there is no odor of petroleum benzine.

**INSECTS AND INSECT FRAGMENTS; RODENT HAIRS AND EXCRETA****42.27*****Large Quantities of Bran or Chocolate Absent***

Weigh 225 g of sample into 1.5 liter beaker. Add 50 ml of pancreatin soln, 42.2(c), diluted in sufficient H<sub>2</sub>O to saturate sample and leave some free liquid. Stir until lumps are disintegrated. Adjust to pH 7-8 with ca saturated Na<sub>3</sub>PO<sub>4</sub> soln. Allow to stand ca 15 min., and if necessary readjust to pH 7-8. Maintain at 40° for not less than 3 hours. Dilute to an easily stirred mixture. Acidify with HCl to pH 6-6.5. With stirring, boil 5 min. or heat on steam bath 30 min.

(a) *Fruit, nuts, and coconut absent.*—Filter with suction thru No. 80 sieve or comparable silk bolting cloth. Rinse with H<sub>2</sub>O. Refilter liquid thru another sieve or cloth, using minimum amount of suction. Examine sieves or cloths microscopically.

(b) *Fruit, nuts, and coconut present.*—Pour digested mixture thru No. 40 metal sieve of size suitable to quantity of residue, and wash residue with H<sub>2</sub>O. Filter liquor and washings as directed under (a) and examine. Transfer residue remaining on the No. 40 metal sieve to Wildman trap flask, 42.1(a). Use 500 ml, liter, or 2 liter trap flask according to quantity of residue, and mix with 15, 30, or 50 ml of gasoline. Fill with 40% alcohol. Trap off after 30 min., filter, and examine microscopically. Transfer residue in trap flask, all or little at a time, to white enameled pan, cover with small quantity of H<sub>2</sub>O, and examine for rodent excreta.

**42.28*****Large Quantities of Bran or Chocolate Present***

Weigh 225 g sample into 1.5 liter beaker. Add 1 liter of boiling 1-2% HCl. Boil until mixture becomes finely divided and so digested that it will not froth over when covered. Neutralize to ca pH 7 with NaOH soln (1+1) and then to pH 7.5-8 with ca saturated Na<sub>3</sub>PO<sub>4</sub> soln. Digest with pancreatin soln, 42.2(c), as directed in 42.27, bring to boiling, cool, transfer to 2 liter Wildman trap, and extract, using gasoline and H<sub>2</sub>O. Trap off onto ruled, rapid-acting filter paper in Hirsch funnel and examine microscopically. If excessive amounts of bran or chocolate rise into neck of flask, transfer trapped-off portion to the Wildman trap flask and re-extract, using 40% alcohol.

**WHITE WHEAT FLOUR****42.29****RODENT EXCRETA**

Weigh 50 g of flour into 250 ml beaker. Add CHCl<sub>3</sub> to within ca 1 cm of top of beaker, mix thoroly, and allow to settle at least 30 min. Several times during this period, stir layer that rises to top. Decant soln and floating flour, being careful not to disturb heavy residue in bottom of beaker. Before decanting, take care that floating layer has not become so compact as to render this operation difficult. Wash down sides of beaker with stream of CHCl<sub>3</sub> from wash bottle. If so much material remains that it would interfere with subsequent microscopic examination, repeat decanting after adding more CHCl<sub>3</sub> until very little flour is left in beaker. Take care not to decant any heavy filth that may be present. Wash heavy residue remaining in beaker with stream of CHCl<sub>3</sub> onto ruled filter paper in Hirsch funnel. Transfer filter paper to Petri dish and examine microscopically.

When procedure 42.30 is to be carried out on same 50 g portion, decant into Büchner funnel fitted with rapid-acting filter paper. Draw air thru funnel until soln stops dripping. Transfer contents of funnel to sheet of clean smooth paper, using spatula to remove particles adhering to filter paper. Dry in air until no odor of  $\text{CHCl}_3$  can be detected, transfer to Wildman trap flask, 42.1(a), and proceed as directed in 42.30.

**42.30****INSECTS, INSECT FRAGMENTS, AND RODENT HAIRS**

Weigh 50 g of flour into 250 ml beaker. Add ca 60 ml of pancreatin soln, 42.2(c), diluted with equal volume of  $\text{H}_2\text{O}$  and stir into smooth paste. Add ca 40 ml of the pancreatin soln (100 ml total) and mix. Adjust pH and digest as directed in 42.27. Transfer digested material to liter Wildman trap flask. Add 20 ml of gasoline and mix thoroly. Allow mixture to stand 5 min., fill with saturated NaCl soln, and after 30 min. trap off into 250 ml beaker. Transfer contents of beaker to the trap flask and fill with saturated NaCl soln. Stir, and after ca 30 min. trap off into beaker and filter thru rapid filter paper, using suction. Examine microscopically, 42.1(j).

**42.31****INSECT EXCRETA (3)**

(a) *Optional for 1-4 samples.*—Weigh 0.20 g of flour on tared, flat, glass disk of 7-7.5 cm diam. Add clove oil and spread mixture into thin uniform layer. (There should be sufficient oil present to clear flour and present smooth surface of oil, but not so much that mixture will flow off disk.) Place wire grid over disk and examine microscopically with dark background and intense reflected light.

(b) *Optional in multiple-sample schedule.*—Tare 2-8 small numbered vials on each balance pan and weigh by shifting weights from one side to other. (If desired, larger portion may be weighed in beaker and some of flour floated off in a  $\text{CHCl}_3$ -ether or  $\text{CHCl}_3$ -toluene mixture, sp. gr. 1.40, before transferring to filter paper.) Rinse contents of each vial onto smooth-surfaced, ruled filter paper in Hirsch funnel with  $\text{CHCl}_3$  or  $\text{CCl}_4$ . Transfer paper to Petri dish, flood with clove oil, and examine with dark background and intense reflected light.

**WHOLE CORN MEAL (4)****42.32****RODENT EXCRETA**

Weigh 50 g portion of sample in 250 ml hooked-lip beaker. Add  $\text{CHCl}_3$  to ca 1 cm of top, mix thoroly, and allow to settle at least 30 min. Several times during this period stir layer that rises to top. Decant  $\text{CHCl}_3$  and floating corn tissue into Büchner funnel, taking care not to disturb heavy residue in bottom of beaker. Before decanting, take care that floating layer has not become so compact as to render this operation difficult. Add quantity of  $\text{CCl}_4$  equal to quantity of  $\text{CHCl}_3$  and corn tissue left in beaker, allow to settle again, and decant as before. Repeat this process with mixture of equal parts of  $\text{CHCl}_3$  and  $\text{CCl}_4$  until very little corn tissue remains in beaker. Wash residue in beaker onto 7 cm ruled filter paper with stream of  $\text{CHCl}_3$  or  $\text{CCl}_4$  and examine microscopically, 42.1(j).

**42.33****INSECTS, INSECT FRAGMENTS, AND RODENT HAIRS**

Draw air thru bulk of material in Büchner funnel, 42.32, until liquid has evaporated. Air-dry on sheet of paper until no odor of  $\text{CHCl}_3$  remains. Transfer residue to liter Wildman trap flask. Add 100 ml of 60% alcohol and mix thoroly. Wash down sides of flask with the alcohol and soak 30 min. Add ca 35 ml of gasoline, mix thoroly, and allow to stand 5 min. Fill with the alcohol, allow to stand 30 min., trap off, and examine as directed in 42.32.

**DEGERMINATED CORN MEAL, CORN GRITS****42.34****RODENT EXCRETA**

Proceed as directed under 42.32, but effect complete separation with  $\text{CHCl}_3$  (*not*  $\text{CCl}_4$ ).

**CREAM CORN MEAL, RYE MEAL, WHEAT MEAL,  
WHOLE WHEAT FLOUR****42.35****RODENT EXCRETA.—See 42.32.****42.36****INSECTS, INSECT FRAGMENTS, AND RODENT HAIRS**

(a) *Cream corn meal*.—Proceed as directed under 42.33, but substitute saturated NaCl soln for 60% alcohol.

(b) *Rye meal*.—Proceed as directed under 42.33, but substitute 40% alcohol for 60% alcohol and light mineral oil for gasoline; also soak material in alcohol 15 min. before adding oil.

(c) *Wheat meal*.—Proceed as directed under (b), except to soak 10 min. instead of 15 min.

(d) *Whole wheat flour*.—Proceed as directed under 42.30, except to substitute 60% alcohol in first trap in place of the saturated NaCl soln, to soak flour in 60% alcohol 10 min. before adding oil, and to substitute  $\text{H}_2\text{O}$  for the NaCl soln in second trap.

**ALIMENTARY PASTES****42.37****FILTH**

Proceed as directed in 42.28, but do not re-extract in 40% alcohol.

**STARCH****42.38****FILTH**

Mix 200 g of starch with cold  $\text{H}_2\text{O}$  and stir out lumps. Dilute with cold  $\text{H}_2\text{O}$  and rinse thru No. 100 sieve or 10XX silk bolting cloth. Rinse particles held on sieve onto filter paper. Examine filter paper or cloth microscopically.

**EGGS—FROZEN OR DRIED****42.39****EXTRANEOUS MATTER**

(a) *Exclusive of metal fragments*.—Weigh 20 g of dried whole egg into 100 ml beaker. Add petroleum benzine to within 1–2 cm of top of beaker and thoroly mix. Allow to stand until powdered egg has settled to bottom and then decant supernatant liquid. Add more petroleum benzine, mix, and decant 4 additional times. Evaporate petroleum benzine remaining. If heat is used, take care that egg is not cooked after most of petroleum benzine is evaporated. Make a paste of the egg with 5% NaCl soln. Add more NaCl soln to thin mixture and transfer to 800 ml beaker. Add 350 ml of ca 12%  $\text{H}_3\text{PO}_4$  and heat to boiling, taking care when boiling starts that egg does not froth over. (By heating over low heat, digestion will have time to proceed before boiling occurs and there will be little foam. A froth can usually be broken by addition of 4 or 5 drops of capryl alcohol. Once froth is broken, egg mixture may boil with even rolling motion or may froth up again. Persistent frothing can be reduced by adding 10–15 drops of mineral oil after breaking foam.) Filter thru ruled, rapid filter paper in Hirsch funnel.

(b) *Exclusive of hairs and feathers.*—Weigh 20 g of egg into 800 ml beaker. Add ca 350 ml of 5% NaCl soln and stir until smooth, then stir in 12–13 ml of 40% NaOH soln. Heat to boiling, stirring frequently during preliminary heating and until mixture starts turning with convection currents. When boiling starts, take care that the egg does not froth over. Boil 5 min., filter thru ruled, rapid filter paper in Hirsch funnel, and examine.

## 42.40

## CHICKEN EXCREMENT

Weigh into 250 ml wide-mouth centrifuge bottle 150 g of liquid whole eggs, yolks, or whites; or 25 g of dried whole eggs or yolks; or 150 g of reconstituted egg albumen. Add 50 ml of 5% NaCl soln to liquid eggs or 150 ml to dried eggs, shake thoroly to form homogeneous mass, and centrifuge 5 min. at 1500 r.p.m. (These are minimum conditions; greater centrifugal force or longer time is satisfactory. Sediment should be gathered solidly at bottom of bottle. Do not brake centrifuge to stop.) Decant supernatant liquid and add 100 ml of 5% NaCl soln to material remaining in centrifuge bottle. Shake well, centrifuge 5 min. at 1500 r.p.m., and decant again. Add 100 ml of saturated NaCl soln to material remaining in centrifuge bottle, shake well, and centrifuge 5 min. at ca 500 r.p.m. Decant the supernatant liquid as above. Repeat this step as many times as necessary in order to remove most of egg material (ordinarily single repetition is sufficient). Filter sediment thru Hirsch funnel fitted with ruled, rapid filter paper, using suction. Use minimum of H<sub>2</sub>O. Transfer paper to Petri dish, keep moistened with H<sub>2</sub>O, and examine at ca 30 diam. Remove any white to yellowish white chalky amorphous-appearing material to depression of spot plate. Add small drop of 1% NaOH soln and stir well, using small glass stirring rod. While stirring, add 5 drops of the NaCN soln, 42.2(e), and 1 drop of arsenophosphotungstic acid soln, 42.2(d). Immediate development of blue color associated with the chalky granules demonstrates presence of uric acid or its salts.

Large amounts of egg material in filtrate will develop blue-green color after standing for 1–2 min. or upon vigorous stirring. However, this reaction differs from the uric acid reaction in that it is delayed and is off color. As metals are attacked by the reagents and give a false test, use of forceps, needles, etc., must be avoided. Similarly metal fragments (often found in dried eggs) may interfere.

## STRAWBERRIES (FROZEN)

## 42.41

## PREPARATION OF SAMPLE

Thaw entire sample representing one barrel or other container and divide into 2 equal portions without damaging berries.

## 42.42

## ROT

(a) *Macroscopic separation.*—Drain all of one portion of sample on No. 20 sieve. Immerse berries in tap H<sub>2</sub>O in large white pan. Decant most of H<sub>2</sub>O thru No. 20 sieve, catching and returning any strawberry tissue. Repeat washing until H<sub>2</sub>O is fairly clear. Examine berries under H<sub>2</sub>O and remove all questionable berries to another pan containing deaerated H<sub>2</sub>O. Re-examine suspected berries. Confirm all questionable rot spots by examining fragment of the berry tissue for mold under compound microscope. Drain separately the good and rotten berries 2 min. on No. 20 sieve. Weigh rotten berries, add to good, and determine total drained weight of sample.

(b) *Mold count*.—Drain second portion of sample on No. 20 sieve 2 min. Pulp drained berries thru cyclone with screen openings ca 0.027" in diam., or thru No. 30 sieve, using stiff brush, and mix thoroly. Mix 50 g of pulp with 50 g of 3% pectin soln, 42.2(b). Make mold count on this mixture as directed under 42.57.

#### APPLE BUTTER

42.43

##### ROT

Thoroly mix each subdivision of sample and make a mold count of each as directed in 42.57.

42.44

##### INSECT AND RODENT FILTH

Weigh out 100 g of sample into 400 ml beaker, add sufficient hot H<sub>2</sub>O to obtain uniform dispersion, and pour into 2 liter Wildman trap flask, 42.1(a). Rinse beaker with hot H<sub>2</sub>O and add rinse H<sub>2</sub>O to flask. Add 25–35 ml of castor oil and sufficient hot H<sub>2</sub>O to bring oil layer into neck of flask. Stir vigorously with vertical motion while adding the H<sub>2</sub>O. Let stand 30 min. Trap off, filter, and examine paper microscopically.

#### DRIED APPLE PRODUCTS

42.45

##### HEAVY FILTH

Weigh out 50 g of sample and place in liter Erlenmeyer flask or other suitable container. Add enough tap H<sub>2</sub>O to cover apples and shake or stir 5 min. Empty contents of flask into No. 6 or No. 8 sieve, recovering H<sub>2</sub>O in beaker. Wash out flask and add rinse H<sub>2</sub>O to that in beaker. Rinse off pieces of apple with fine stream of tap H<sub>2</sub>O delivered with as much force as possible. Catch this rinse H<sub>2</sub>O in beaker also, transfer to 2 liter separator, and allow to stand 15 min., with occasional gentle rotary shaking. Open stopcock and draw off heavy particles and small amount of the liquid. Filter thru rapid-acting paper and examine microscopically.

42.46

##### INSECTS AND LIGHT FILTH

Place 50 g of sample in beaker, cover with H<sub>2</sub>O, and boil 15 min. Empty apples onto No. 6 or No. 8 sieve, recovering H<sub>2</sub>O in beaker. Rinse beaker. Rinse apples with strong stream of hot H<sub>2</sub>O. Place all of H<sub>2</sub>O in Wildman trap flask, 42.1(a), add 20 ml of castor oil, mix well, and add sufficient hot tap H<sub>2</sub>O to fill flask. Allow to stand 30 min. with occasional stirring. Trap off oil layer, add H<sub>2</sub>O to flask, stir, and trap off again in 10 min. Filter trapped-off portion thru rapid paper. Examine microscopically.

#### FRESH, CANNED, AND FROZEN BLACKBERRIES, BLUEBERRIES, GOOSEBERRIES, HUCKLEBERRIES, LOGANBERRIES, RASPBERRIES, AND CHERRIES

42.47

##### ROT

##### *In Canned Blackberries and Raspberries*

Drain contents of No. 2 can or its equivalent on No. 20 sieve (5" diam.). Immerse berries in H<sub>2</sub>O in large white pan. Decant most of H<sub>2</sub>O thru No. 20 sieve and add more H<sub>2</sub>O. Repeat washing if H<sub>2</sub>O is not fairly clear. Examine berries under H<sub>2</sub>O and remove all questionable berries to black-bottomed pan containing deaerated H<sub>2</sub>O. Re-examine suspected berries and note particularly outline of each berry as it is turned over under strong beam of light. Separate into two classes—those with ex-

ternal mold, and those moldy but without external mold. Confirm all questionable rot spots by examining fragment of tissue for mold under compound microscope.

Drain separately the good, and the 2 separations of rotten berries and fragments, for 2 min. on No. 20 sieve (5" diam.). Weigh each rotten portion separately and add to good berries and weigh.

If sample contains large amount of disintegrated berry material, pick out whole berries and large fragments. Remove disintegrated material to No. 20 sieve and allow it to drain 2 min. and then weigh. Take aliquot of ca one-fourth and separate rotten from good material. Calculate total weight of rot in all disintegrated material and add this to weight of whole berries showing internal mold. Mix all portions together, drain, and determine total drained weight of sample. Calculate and record percentages of material with external and internal mold. Pulp berries thru cyclone with openings ca 0.027" in diam., or thru No. 30 sieve, using stiff brush. Mix pulp thoroly, weigh 50 g, and dilute with equal weight of 3% pectin soln, 42.2(b). Make mold count on this mixture as directed in 42.57.

### INSECTS

#### 42.48 *In Blueberries, Gooseberries, Huckleberries, Loganberries, Raspberries, and Cherries*

Add ca 400 ml of H<sub>2</sub>O to 500 g of fruit in large container (e.g., No. 10 can). Stir thoroly and pick out any larvae that may rise to surface. If fruit is uncooked, boil 30 min., adding sufficient H<sub>2</sub>O to maintain water level. Transfer fruit to No. 6 sieve and pass drained liquid thru No. 40 sieve, or cloth with same opening size. Crush material on the No. 6 sieve and rinse it and released material into 2 liter Wildman trap flask, 42.1(a). Cool to room temp. and mix in 25 ml of gasoline. Fill flask with H<sub>2</sub>O and allow to stand 30 min., trap off onto same No. 40 sieve or cloth, and examine microscopically.

(a) *Alternative Method.*—Place 500 g of fruit on No. 8 sieve, catching liquid that passes thru. Pass liquid thru No. 40 sieve or cloth and examine for insects. Crush fruit, rinse sieve, and add drippings, crushed fruit and rinse H<sub>2</sub>O to large container (e.g., No. 10 can), and boil 30 min. Cool to room temp., add ca 100 ml of gasoline or kerosene to can, and stir vigorously. Add H<sub>2</sub>O to container until liquid surface is within 1–2 cm of top. Allow mixture to come to rest and collect any floating insects. Repeat stirring 8–10 times, collecting insects after each stirring.

#### 42.49

#### *In Blackberries*

Weigh out 500 g of berries and pulp thru No. 6 sieve under H<sub>2</sub>O. Add to pulp any debris remaining on sieve. Pour mixture onto No. 60 sieve, drain off most of H<sub>2</sub>O, and discard. Stir pulp during draining. Transfer with H<sub>2</sub>O to 2 liter Wildman trap flask, 42.1(a), pulp remaining on sieve. Add H<sub>2</sub>O to ca 2" above level of pulp in flask. Add 25 ml of HCl and boil 30 min. Direct stream of air into flask to prevent foaming. Cool mixture to room temp. and add 40 ml of kerosene. Mix thoroly 2 min., but not so vigorously that emulsion forms. Fill flask with H<sub>2</sub>O and let stand 30 min. with occasional stirring. Trap off, filter, and examine microscopically.

### MAGGOTS

#### 42.50

#### *In Blueberries*

Weigh 567 g (20 oz.) of fresh berries or use No. 2 can of processed berries. Add 100 ml of H<sub>2</sub>O to fresh or frozen berries and boil 5 min. with frequent stirring. (Omit

this step if berries are canned.) Transfer 1 cm layer of berries to No. 6 sieve immersed in pan of H<sub>2</sub>O. Shake loose maggots and debris thru sieve. Mash berries carefully under H<sub>2</sub>O in order to rub any remaining maggots thru sieve. Rinse, and discard pulp. Repeat above process with another portion of berries. After all berries have been screened, decant ca  $\frac{3}{4}$  of H<sub>2</sub>O from pan. Add more H<sub>2</sub>O and again decant several times to remove as much debris as possible without losing maggots. Transfer residue to black-bottomed pan and count maggots by thoroly examining all contents of pan.

**42.51***In Cherries*

Weigh 567 g (20 oz.) of fresh cherries or use No. 2 can of processed cherries. Crush or mash raw cherries and boil 5 min. with stirring. (Omit this step if the cherries are canned.) Transfer cherries to No. 4 sieve resting in 2" of H<sub>2</sub>O in large pan. Rinse out can or cooking pan onto sieve. Rub cherries thru sieve until only skins remain. Agitate sieve under H<sub>2</sub>O to release any trapped maggots and discard skins and seeds. Decant H<sub>2</sub>O and coarse pulp slowly from pan. Add more H<sub>2</sub>O and repeat. Transfer residue to No. 6 sieve resting in 1" of H<sub>2</sub>O in black-bottomed pan. Shake sieve until maggots have dropped thru, and discard pulp. Decant H<sub>2</sub>O from pan until layer is  $\frac{1}{2}$ – $\frac{3}{4}$ " deep. Stir contents of pan with circular motion and count maggots by carefully examining all contents of pan.

**FIG AND PRUNE PASTE****42.52****LIGHT FILTH**

Place 100 g of sample in beaker with ca 300 ml of H<sub>2</sub>O. Boil and stir until paste is thoroly softened and mix with H<sub>2</sub>O. Transfer to 2 liter Wildman trap flask, 42.1(a), add 20 ml of castor oil, and mix thoroly. Add sufficient hot tap H<sub>2</sub>O to fill flask and allow to stand at least 30 min. with occasional stirring. Trap off oil layer. Add H<sub>2</sub>O to flask, stir, and trap off again after 10 min. Filter trapped-off portions thru rapid paper and examine microscopically.

**MINCE MEAT****42.53****FILTH**

Weigh 100 g sample into 400 ml beaker. Add 150 ml of hot H<sub>2</sub>O and stir. Pour mixture from beaker onto 100-mesh screen and wash well with hot H<sub>2</sub>O to remove excess sugar, which prevents absorption of alcohol. Place in liter Wildman trap flask, 42.1(a). Add 200 ml of 80% alcohol. Boil 5 min. Cool to room temp. Add 50 ml of gasoline, mix thoroly, and after allowing to stand 5 min. fill flask with 80% alcohol. Trap off, filter, and examine.

**CANDY****42.54****FILTH**

Examine at least 100 g, and with assorted candies examine each variety separately.

(a) *In hard candy readily soluble in hot H<sub>2</sub>O.*—Dissolve in hot H<sub>2</sub>O in 1500 ml beaker. Filter thru 7 cm rapid filter paper in Büchner funnel. Examine filter for filth.

(b) *In candy with gums, starches, albumen, etc.*—Dissolve in boiling 0.5% HCl soln. Filter thru 7 cm rapid filter or 10 cm disk of bolting cloth (ca 100-mesh) in suction

funnel. Examine filter for filth. If albumen interferes with filtration, add 1 or 2 g of pepsin in filtered soln to the acid soln of candy. Allow to stand 2 hours at 50°. Heat to boiling and filter.

(c) *In chocolate candy without fruit or nuts.*—Dissolve in large quantity of  $\text{Na}_2\text{B}_4\text{O}_7$  soln, saturated at room temp. and heated to boiling. Simmer 10–15 min. Filter with or without suction thru No. 100 sieve or its equivalent. Wash sieve upside down in funnel or evaporating dish and transfer filth to 7 cm filter paper. Use  $\text{H}_2\text{O}$  under pressure from a spray nozzle if convenient. The sieve may be replaced by a 10 cm disk of 100-mesh bolting cloth in a 7 cm suction funnel. Examine the bolting cloth directly for filth if it is relatively free from chocolate particles.

If residue in evaporating dish contains cocoa shell or other material that would obscure the filth on the paper if filtered, transfer this residue to 1 or 2 liter Wildman trap flask, 42.1(a). Add 25–35 ml of gasoline, mix thoroly, and allow to stand 5 min. Fill flask with  $\text{H}_2\text{O}$  and make extractions as directed in (d).

(d) *In chocolate candy with fruit or nuts.*—Dissolve in  $\text{Na}_2\text{B}_4\text{O}_7$  soln as directed in (c). Filter thru No. 10 sieve in combination with the No. 100 sieve or bolting cloth. Wash material on the No. 10 sieve thoroly with stream of  $\text{H}_2\text{O}$  to transfer any adhering insects or rodent hairs to the No. 100 sieve. Transfer filth from the No. 100 sieve to filter paper as directed in (c). (If fruit or nut particles are small, it may be necessary to use No. 20, 30, or even No. 40 sieve.) Wash material remaining on the No. 10 sieve into a liter Wildman trap flask with 40% alcohol. Cover nuts with the alcohol and boil 5 min. Cool to room temp. Add 25–35 ml of kerosene or gasoline, and mix thoroly, but avoid an emulsion. Fill the flask with 40% alcohol. Allow to stand 30 min., stirring every 5 min. Trap off and filter thru 7 cm rapid filter. If first extraction yields filth, add to flask 20 ml of kerosene or gasoline and stir vigorously. Allow to stand 10 min. and trap off. Pour off liquor remaining in trap flask. Transfer residue to flat white pan and examine for rodent pellets.

(e) *In fruit and nut candy without chocolate.*—Dissolve and filter the candy as directed in (d). Use filter paper in place of the No. 100 sieve in combination with one of the other sieves of larger mesh.

(f) *In black candy containing carbon.*—Dissolve candy by boiling in 0.5%  $\text{HCl}$ . Transfer to Wildman trap flask and cool to room temp. Add 35 ml of kerosene or gasoline and mix thoroly. Fill flask with  $\text{H}_2\text{O}$  and allow to stand 10 min. with almost constant stirring. Trap off and filter. As alternative method, omit use of the trap flask, pour soln thru No. 100 sieve, and transfer filth to filter paper as directed in (c).

## SUGARS

### 42.55

#### FILTH

Dissolve 100 g in ca 200 ml of hot  $\text{H}_2\text{O}$ . Boil, and filter at once thru rapid filter paper in Büchner funnel. Examine filter microscopically.

## SIRUPS, MOLASSES, AND HONEY

### 42.56

#### FILTH

Mix sample thoroly and dissolve 200 g in 200 ml of hot  $\text{H}_2\text{O}$  acidified with 5 ml of  $\text{HNO}_3$ . Filter at once thru 7 cm rapid filter in suction funnel. Wash with minimum of hot  $\text{H}_2\text{O}$  and examine microscopically for filth.

(a) *Alternative procedure.*—Dissolve 200 g in 500 ml of hot  $\text{H}_2\text{O}$ . Filter at once thru 100-mesh bolting cloth in suction funnel. Wash with minimum of hot  $\text{H}_2\text{O}$  and examine as directed above.



## TOMATO PRODUCTS (NOT DEHYDRATED)

## 42.57

## MOLDS (δ)—OFFICIAL

In making mold counts of tomato products, use juice and catsup as it comes from container. If necessary add clean, mold-free, water-soluble gum to assist in making more uniform mounts; in case of purée and paste mix  $H_2O$  to make total tomato solids of diluted product 8.37–9.37%.

Clean the Howard cell, 42.1(c), so that Newton rings are produced between slide and cover-glass. Remove cover and place small drop of well-mixed sample upon central disk; using knife blade or scalpel, spread drop evenly over disk, and cover with glass so as to give an even spread. Use only sufficient sample to bring material to edge of disk. (It is of utmost importance that the drop be taken from a thoroly mixed sample and spread evenly over slide disk. Otherwise, when cover slip is put in place, insoluble material, and consequently molds, may be more abundant at center of mount.) Discard any mount showing uneven distribution or absence of Newton rings, or liquid that has been drawn across moat and under cover-glass.

Place slide under microscope and examine with such adjustment that each field of view covers 1.5 sq. mm. (This area, which is of vital importance, may frequently be obtained by so adjusting draw-tube that diameter of field becomes 1.382 mm. When such adjustment is not possible, make an accessory drop-in ocular diaphragm with aperture accurately cut to necessary size. Diameter of area of field of view can be determined by use of stage micrometer. When instrument is properly adjusted, quantity of liquid examined per field is 0.15 cu. mm.)

From each of two or more mounts examine at least 25 fields taken in such manner as to be representative of all sections of mount. Observe each field, noting presence or absence of mold filaments and recording results as positive or negative, as case may be. (No field should be considered positive unless aggregate length of not more than 3 of filaments present exceeds  $\frac{1}{4}$  of diameter of field.) Calculate proportion of positive fields from results of examination of all observed fields and report as percentage of fields containing mold filaments. In case greater accuracy is desired more fields should be counted.

## 42.58

## YEASTS AND SPORES—OFFICIAL

Fill graduated cylinder with  $H_2O$  to 20 ml mark and add sample till level of mixture reaches 30 ml mark, to obtain a dilution of 1:3. Close graduate, or pour contents into Erlenmeyer flask, and shake vigorously 15–20 seconds. To assure thoro mixing, have mixture fill not more than  $\frac{3}{4}$  of container in which shaking is performed. For tomato sauce or pastes, or products running high in number of organisms or of heavy consistency, use dilution that permits ready counting.

Pour mixture into beaker. Thoroly clean blood-counting cell, 42.1(e), to obtain good Newton rings. Thoroly stir contents of beaker with scalpel or knife blade and after allowing to stand 3–5 seconds remove small drop, place it upon central disk of the blood-counting cell, and cover immediately with cover-glass. Discard any mount showing uneven distribution, absence of Newton rings, or liquid that has been drawn across moat and under cover-glass. Allow slide to stand not less than 10 min. before beginning to make the count. Use magnification of 300–400 diameters. Count number of yeasts and spores in either whole counting area (1.0 sq. mm.), which represents volume of 0.1 cu. mm., or portion thereof. If less than whole volume is counted, choose areas for counting that are representative. Calculate to number of yeasts and spores/ml or g of original products.

Example: 25 spores were observed in  $\frac{1}{5}$  of the 1.0 sq. mm. area, and dilution of 4 parts of H<sub>2</sub>O with 1 part of product was made originally. Then total number of organisms =  $25 \times 2 \times 10 \times 1000 \times 5 = 2,500,000$ , where

- 25 = No. of organisms observed;
- 2 = factor to determine No. of organisms/0.1 cu. mm.;
- 10 = factor to determine No. of organisms/1.0 cu. mm.;
- 1000 = factor to determine No. of organisms/1.0 ml; and
- 5 = factor representing dilution.

**42.59****ROT FRAGMENTS (6)**

Weigh 10 g of juice (5 g of purée or catsup or 2 g of paste) and transfer with 100 ml of H<sub>2</sub>O to 400 ml beaker. Add ca 2 ml of saturated aqueous gentian violet soln (10% gentian violet in alcohol may replace the aqueous soln when latter fails), stir, and allow to stain 3 min. Add ca 200 ml of H<sub>2</sub>O, stir, and pour thru No. 60 sieve ca 7.5 cm in diam., held in horizontal position. Pour material over entire surface of sieve, using glass rod held against lip of pouring beaker, with lower end of rod ca 2 cm from screen. If sample weight specified does not drain rapidly, reduce size of sample. Rinse beaker with 200 ml of H<sub>2</sub>O and pour rinse H<sub>2</sub>O over tomato debris on sieve, using glass rod as before. Tilt sieve to ca 30° angle and wash debris to lower part with ca 100 ml of H<sub>2</sub>O. Allow debris to drain and transfer to bottom of graduated tube, ca 12×3 cm, by means of spatula and rinsing. (Usually 300 ml of rinse H<sub>2</sub>O will be ample for complete transference of tomato debris.) When completely transferred, make volume of H<sub>2</sub>O and debris to 10 ml with H<sub>2</sub>O. Add sufficient neutralized 1% algin soln, 42.2(b) (2), to make volume to 20 ml. Mix stained suspension well, measure out two separate 0.5 ml portions, spread over 2 counting plates, 42.1(d), and cover with special cover slip. Examine each slide with Greenough-type microscope, 42.1(j), using magnification of 40–45 diam. with transmitted light. (Rot fragments are tomato tissue to which mold filaments are attached. Some may appear as almost solid masses of mold.) Count number of rot fragments on each of the 2 slides, add, and multiply by 2 (10 g sample), 4 (5 g sample), or 10 (2 g sample) to obtain number of rot fragments/g of product.

**42.60****ROT IN CANNED TOMATOES**

Drain contents of can 2 min. on No. 2 sieve. For containers of less than 3 lbs. net weight, use sieve 8" in diam.; for containers of 3 lbs. or more net weight, use 12" sieve. Examine drained tomatoes and record number and size of any rotten portions present. Run drained tomatoes thru laboratory cyclone with screen openings ca 0.027" in diam., or brush thru No. 30 sieve with stiff-bristle brush. Make mold counts on both drained juice and pulped tomatoes as directed in 42.57.

**42.61****FLY EGGS AND MAGGOTS**

(a) *In comminuted products.*—Thoroughly mix sample and transfer 100 g to 2 liter separator. Add 20–30 ml of gasoline and thoroughly shake contents of separator, releasing pressure as necessary. Fill separator with H<sub>2</sub>O in such manner as to produce maximum agitation. Place separator and contents in ring stand and allow to settle; at 15 minute intervals gently shake contents of separator with rotary motion to facilitate settling out of fly eggs and maggots. At end of 1 hour open stopcock and drain out 50–75 ml of bottom liquid. If this material contains seeds, pass it thru No.

10 sieve, and rinse seeds and sieve thoroly, recovering both the liquid portion and the rinse  $H_2O$  in a beaker. Filter liquid thru a 10XX bolting cloth in Hirsch funnel, taking care that liquid does not rise above edge of cloth. Examine cloth for eggs and maggots with magnification of ca 10 diam. If fly eggs or maggots are found, continue rotary shaking of separator and trap off another portion at end of 1 hour; examine as before.

(b) *In canned tomatoes*.—Pulp entire contents of can in such a way that minimum number of eggs and maggots are crushed or broken. (This may be done by passing material thru No. 6 or No. 8 sieve and adding seeds and residue remaining on sieve to the pulp.) Place 500 g of the well-mixed pulped tomatoes in 5 liter separator. Add 125–150 ml of gasoline and ca 1 liter of  $H_2O$  and shake contents of separator vigorously, releasing pressure as necessary. Fill separator with  $H_2O$ . Place separator and contents in ring stand and allow to settle at least 1 hour. At 15 minute intervals gently shake contents of separator with rotary motion to facilitate settling out of fly eggs and maggots. At end of 1 hour open stopcock, drain out 100–125 ml of bottom liquid, pass thru No. 10 sieve, and rinse seeds and sieve thoroly, recovering both liquid portion and rinse  $H_2O$  in beaker. Filter liquid thru 10XX bolting cloth in Hirsch funnel, taking care that liquid does not rise above cloth. Examine cloth for eggs and maggots with magnification of ca 10 diam.

If fly eggs or maggots are found, continue rotary shaking of separator and trap off another portion at end of another hour, examining as before.

#### 42.62

#### INSECT FRAGMENTS (7)

Place 200 g of any tomato product except paste (where 100 g is used) in a Wildman trap flask, 42.1(a), with 20 ml of castor oil and mix well. Add sufficient warm tap  $H_2O$  (ca 50°) to fill flask. (At first bubbles of air have tendency to bring up tomato tissues, but after several stirrings these begin to settle out leaving water layer near the oil fairly clear.) Let stand with occasional gentle stirring 30 min., then trap off oil and water layer into beaker. Wash out neck of flask with alcohol to remove adhering castor oil. Add to flask a little more hot  $H_2O$ , stir, let stand 10 min., and then trap off again to secure any fragments that may have escaped previous trapping. (Occasionally it may be necessary to return trapped-off material to another trap flask and rewash to eliminate tomato tissue, but this procedure is seldom necessary.) Filter trapped-off portion, washing beaker, sides of funnel, and paper thoroly with alcohol to dissolve oil and speed filtration. Examine paper microscopically at 20–30 diameters.

#### 42.63

#### SAND

Weigh 100 g of well-mixed sample into 2–3 liter beaker, nearly fill beaker with  $H_2O$ , mix contents thoroly, and allow to stand 5 min. Decant supernatant liquid into second beaker. Refill first beaker with  $H_2O$  and again mix contents. After 5 min. decant second beaker into third and first into the second; refill and again mix the first. Continue this operation, decanting from third beaker into sink until lighter material is washed from sample. Collect sand from the 3 beakers on weighed Gooch crucible, dry, ignite, and weigh.

Under "Sand" only figure obtained by above method should be reported. Results obtained by the determination of ash insoluble in HCl are not applicable to determination of sand, as sand is so unevenly distributed that reliable results can be obtained only by taking larger sample than is possible in determination of ash.

**TOMATO SOUP, CANNED SPAGHETTI, PORK AND BEANS, AND SIMILAR PRODUCTS CONTAINING TOMATO SAUCE****42.64****MOLD**

(a) *Tomato soup with or without cream*.—Place can in hot  $H_2O$  and heat until contents are thoroly warmed; then open. Transfer 10 ml of thoroly mixed soup to 50 ml centrifuge tube and add 3 ml of KOH soln (1+1). Stir until starch in soup has been dissolved and tissues cleared. Add sufficient  $H_2O$  to fill tube, and centrifuge. (Time required to centrifuge sample varies greatly. With centrifuge arm length of 5½" and at speed of ca 1600 r.p.m., ca 20 min. is required for average sample. In heavy soups the gelatinizing of so much starch sometimes interferes with proper settling out of solids during centrifuging. If liquid remains cloudy it may be necessary to discard sample and start again with only 5 ml of soup and proceed with the usual 3 ml of KOH.) When supernatant liquid is clear, pour it off. If it is not entirely clear, check supernatant liquid for mold before discarding. Add enough  $H_2O$  to residue in tube to bring to original volume of soup, mix, and count mold as directed in 42.57.

(b) *Pork and beans, spaghetti with tomato sauce, spaghetti with meat balls or meat, ravioli, chili con carne, and tamales*.—Place unopened can in hot  $H_2O$  and heat until contents are thoroly warmed. Open can and transfer contents onto No. 6 sieve. Drain until major portion of liquid part has passed thru. (With some products sauce runs thru at once, but in case of some beans and spaghetti 10 or more minutes may be required.) Mix sauce thoroly, place 10 ml in centrifuge tube, and proceed as directed in (a). Use care in counting products containing meat so as not to confuse mold filaments and muscle fibers that bear a superficial resemblance to each other; muscle fibers are usually much thicker and striations are often visible.

(c) *Sardines or other fish with tomato sauce*.—Heat can in boiling  $H_2O$  and drain off sauce as directed in (b). Mix sauce thoroly and place portion in centrifuge tube. Centrifuge until oil rises to surface. Discard oil and treat 10 ml of well-mixed sauce with 3 ml of KOH soln as directed in (a). Use care to differentiate between mold filaments and muscle fibers from the fish. Count as directed in 42.57.

**DEHYDRATED TOMATO PRODUCTS****42.65****MOLD**

(a) *Tomato juice cocktail*.—If possible, ascertain formula used in making the dehydrated cocktail, and from it calculate approximate percentage of tomato solids. Multiply this percentage by 34.36. Resulting figure will be ml of solvent to be used to dilute 2 g of the dehydrated cocktail in preparation for mold counting. (Dilution factor is based on tomato solids content of 5.5% for tomato juice.)

Weigh 2 g of well-mixed sample into 50 ml beaker and add 15 ml of  $H_2O$ . Determine weight of sample, including beaker and stirring rod, before any moisture is lost by heating. Heat mixture on steam bath 10–15 min. Weigh beaker and add  $H_2O$  to replace moisture lost by heating. Add sufficient 4% pectin soln to make up difference between 15 ml and calculated volume of liquid needed for the dilution. Mix thoroly and proceed as directed in 42.57.

If formula or tomato solids content of product is not available, make dilutions for mold counting by adding quantity of  $H_2O$  and the pectin soln (half and half) equal to amount of liquid specified in directions for preparing product for consumption. Dissolve flakes as directed above and proceed as directed in 42.57.

(b) *Tomato flakes and tomato soup flakes*.—Ascertain if possible formula used in

making the flakes, and from it calculate approximate percentage of tomato solids. Multiply this percentage by 21.88. Resulting figure will be ml of chloral hydrate soln needed to dissolve 2 g of the dehydrated product in preparation for mold counting. (Dilution factor is based on tomato solids content of 8.37% for tomato purée.)

If product is tomato flakes containing added starch but no added water-soluble solids, determine approximate total tomato-solids content of dry mixture by testing definite dilution with refractometer and converting reading to tomato solids. If reading is in soluble solids, make correction for insoluble tomato solids, which are ca 1% in cyclone juice. (Soluble solids for cyclone juice average ca 4.7%.)

Weigh 2 g of well-mixed sample in 50 ml beaker and add calculated volume of chloral hydrate soln (1+1). Determine weight of sample, including beaker and stirring rod, before any moisture is lost by heating. Boil mixture gently, stirring 5 min. or until it has gelatinous appearance. (Too much clearing of mixture makes it difficult to see the mold.) Weigh beaker and add H<sub>2</sub>O to replace lost moisture. Mix thoroly, and if the mixture remains milky heat almost to boiling, stirring constantly. Proceed as directed in 42.57.

If the formula or tomato-solids content of product cannot be ascertained, dilutions for mold counting may be made on basis of 80% tomato solids for tomato flakes and 40% tomato solids for tomato soup flakes.

### PURÉED INFANT FOOD

#### 42.66

#### ROT

(a) *Mold count.*—Proceed as directed in 42.57. Add ca 0.2 g of NaOH to ca 6 g of product before counting, and stir thoroly until NaOH is dissolved.

(b) *Rot fragments.*—Weigh 1.0 g of puréed fruit into 50 ml beaker. Add ca 20 ml of H<sub>2</sub>O and 0.5–1.0 ml of saturated aqueous gentian violet soln and stain product ca 3 min. Decant thru 10XX silk bolting cloth or thru No. 100 sieve; rinse contents of sieve with 100–200 ml of H<sub>2</sub>O and transfer retained pulp to graduated cylinder. (Transfer is most readily accomplished by rinsing pulp to one edge of sieve, spooning out most of pulp with spatula, and then rinsing sieve with H<sub>2</sub>O from pipet or wash bottle.) Do not transfer more than 10 ml of H<sub>2</sub>O to tube during this operation. Fill to 10 ml mark with H<sub>2</sub>O and then with 1% algin soln, 42.2(b)(2), to 20 ml mark; mix, and measure out two 0.5 ml portions to each of 2 counting plates, 42.1(d), and examine each plate, using Greenough-type microscope, 40–45 diam. magnification, and transmitted light. Count number of rot fragments/slide and calculate and report number/g of product. Rot fragments are identified by presence of mold in or on the plant tissue.

#### 42.67

#### INSECTS AND RODENT FILTH

Utilize the oil-in-H<sub>2</sub>O extraction principle as carried out in Wildman trap flask, 42.1(a). Analyze all foods in similar manner, except to vary temp. and type of oil used as indicated in table.

Transfer contents of 2 cans or jars of food to liter Wildman trap flask previously rinsed with H<sub>2</sub>O. Thoroly mix in ca 20 ml of the oil. Fill with deaerated H<sub>2</sub>O either at room temp. or at 50–70°. Allow mixture to stand 30 min., stirring 4–6 times during this period to release filth from layer of food. Trap off and examine at 20–30 diameters.

Use type of oil and temp. indicated in following table:

<i>Food</i>	<i>Oil</i>	<i>Temperature</i>
All fruits Asparagus Beets Carrots Green beans Peas	Light mineral oil	Room
Spinach	Light mineral oil	50–70°
Squash	Castor oil	50–70°

## 42.68

## FLY EGGS AND MAGGOTS

Transfer residue in the Wildman trap flask (42.67) to 2 liter separator. Add ca 100 ml of gasoline and shake vigorously. Allow material to settle out for ca 2 hours; several times during this period stir layer that rises to top to permit any eggs and maggots to settle out. Withdraw ca 200 ml from bottom of separator and filter this material thru 10XX bolting cloth, using several cloths if there is large accumulation of sediment. Examine microscopically at 15–20 diameters.

## PEAS AND BEANS

## 42.69

## WEEVILS

*Microscopic examination.*—If the peas or beans are canned and of normal texture, pour on No. 8 sieve in pan filled with sufficient H<sub>2</sub>O to stand 2–3 cm deep above mesh of sieve. Mash peas thru sieve with fingers. After as much as possible of material has been worked thru, remove sieve from pan and shake excess H<sub>2</sub>O back into pan. Transfer material retained on sieve to No. 10 can. Pour material that passed thru No. 8 sieve onto No. 40 sieve, discarding that which passes thru. Allow material on sieve to drain few minutes, and shake lightly to remove free H<sub>2</sub>O from solid material. (In case peas are unusually hard, or have tough skins, pass contents of can thru meat or food chopper directly onto No. 40 sieve.) Discard any excess H<sub>2</sub>O passing thru this sieve. Cook dried or frozen peas before maceration.

Add material retained on the No. 40 sieve to the No. 10 can. Add to this material ca 130 ml of gasoline and mix thoroly with large spoon. Rinse into can with H<sub>2</sub>O any remnants of material remaining on sieve. Stir material in can and pick out any insects that may rise to top of water layer. Repeat stirring and searching several times until no more larvae are recovered by this procedure.

Add enough H<sub>2</sub>O to bring contents of can to within 1–2 cm of top. Pick out any larvae visible at surface. Stir again, allow mixture to stand ca 5 min. and then skim off with spoon the gasoline and upper part of H<sub>2</sub>O layer and place in Wildman trap flask, 42.1(a), that has been filled ca quarter full of H<sub>2</sub>O. Add 90–100 ml of gasoline to material remaining in No. 10 can, stir vigorously, and after allowing material to stand for ca 5 additional minutes, skim off gasoline and upper part of water layer as before and add to the material already in trap flask.

Fill flask with H<sub>2</sub>O. Trap off as much as possible of gasoline and filter into Hirsch or Büchner funnel. Lower stopper into flask, and to rinse sides of trap flask apply vacuum ca 5 min. by fitting large rubber stopper and glass tube over mouth of flask. (As an ordinary Erlenmeyer flask will collapse under vacuum of 20" of Hg, use either less vacuum or heavy-walled flask.) Release vacuum, add H<sub>2</sub>O, and trap off. Add trapped portion to that already on filter. Examine microscopically.

**CANNED GREENS AND BROCCOLI****42.70****INSECTS**

Transfer contents of can to pan of suitable size and chop up leaves into pieces 1-2" in length. Weigh out 100 g of well-mixed sample into liter beaker. Add 500-600 ml of H<sub>2</sub>O and boil 5 min. Pour H<sub>2</sub>O and sample into 2 liter Wildman trap flask, 42.1(a). Add 100 ml of gasoline and stir mixture thoroly to insure contact between gasoline and all portions of leaves. Fill flask with deaerated H<sub>2</sub>O, allow to stand 30 min., trap off gasoline layer, filter, and examine. Add 40 ml of gasoline to flask and repeat extraction.

To overcome difficulty encountered by leaves occasionally rising to gasoline-H<sub>2</sub>O interfac, place No. 8 sieve, 6-8" in diam., in suitable sized evaporating dish containing sufficient H<sub>2</sub>O to cover screen ca ½". Pour entrapped gasoline from trap flask onto sieve as it is held under the H<sub>2</sub>O. Move sieve gently up and down to allow aphids to pass thru into the H<sub>2</sub>O. Remove screen and filter contents of dish. Repeat washing procedure to free any aphids left on greens on screen and filter washings. Examine filter papers.

**42.71****HEAVY FILTH**

Recover heavy filth such as soil, maggots (especially those of the spinach leaf miner) and rodent excreta, that sink to bottom of trap flask by following procedure: Transfer contents of trap flask by rinsing with H<sub>2</sub>O into 4-6 quart pail. Add H<sub>2</sub>O to pail until ca full. Stir material, allow to stand short time, and decant ca half pail contents. Refill pail with H<sub>2</sub>O and repeat operation until most of floating greens are removed. Wash into black shallow pan the heavy filth left in pail and examine visually for larvae, stones, and other debris, picking material out with pair of forceps.

**CANNED ASPARAGUS****42.72****INSECTS**

Insert 7" glass funnel containing 5" No. 12 sieve into 2 liter Wildman trap flask, 42.1(a). Pour liquid contents of can under examination thru sieve into flask. Wash out can and contents once with H<sub>2</sub>O, keeping asparagus in can. Transfer asparagus to clean white pan and count pieces. Cover asparagus with H<sub>2</sub>O and examine for beetle infestation or damage. Stir to wash stalks and then transfer washings to flask thru sieve. Repeat washing, add 25 ml of kerosene or gasoline to flask, mix well, trap off, and filter. Repeat flotation, using 15 ml of gasoline or kerosene. If small pieces of asparagus float up with oil, remove with forceps and rinse adhering filth from them onto filter. Examine microscopically at 20-30 diameters.

**MUSHROOMS***Canned***42.73****DECOMPOSED MATERIAL**

Empty contents of can into large white pan and cover with H<sub>2</sub>O. Examine each piece macroscopically. Pick out all pieces with decomposed areas. (These areas appear as muddy brown or black discolored patches; areas of more advanced decay are characterized by sunken circular spots with dark-colored centers and lighter-colored edges.) Determine drained weight of decomposed pieces and drained weight of passable material.

42.74

## FILTH

Place all of the mushrooms on No. 6 sieve in deep evaporating dish. Add H<sub>2</sub>O, including that from the white pan, 42.73, so as to practically half immerse sieve. Thoroly break up mushroom pieces by rubbing over sieve. Agitate sieve while under H<sub>2</sub>O, remove sieve, and filter liquid thru rapid paper. Examine filter paper or papers microscopically at 20–30 diameters.

*Dried*

42.75

## FILTH

Thoroly mix sample and weigh out 100 g portion. Transfer mushrooms to Wildman trap flask, 42.1(a), add H<sub>2</sub>O, and allow to soak several hours, preferably overnight. After soaking, add 30 ml of gasoline and churn contents by hard, rapid pounding of mushrooms against bottom of flask, using vertical movement of the rubber plunger. Use regular Wildman trap method for trapping off gasoline layer and filtering. (Second trapping off of contents is necessary to assure that all insects that will float to top in the gasoline are recovered.) Pour remaining liquid and mushrooms in flask onto No. 8 screen in dishpan and add enough H<sub>2</sub>O to partially immerse screen. Rub mushrooms over screen to release any insects, such as maggots, that may have remained in tunnels in the mushrooms. Decant off liquid in dishpan and by usual pick-out method recover maggots present.

## BRUSSELS SPROUTS

42.76

## INSECTS

Count out 24 Brussels sprouts (ca 250 g) and weigh. Slice heads longitudinally into quarters and immerse in H<sub>2</sub>O in white pans. Spread leaves to expose insects, and count those on the heads or falling off into the H<sub>2</sub>O.

## SWEET CORN

42.77

## INSECT FILTH

Make macroscopic examination of whole can, or equivalent of No. 2 can where larger sized can is being examined. Determine number of worm-eaten kernels and of rotten kernels.

Place 200 g of well-mixed sample in 2 liter Wildman trap flask, 42.1(a), with 20 ml of castor oil and mix well. Add sufficient hot tap H<sub>2</sub>O (ca 50°) to fill flask. Let stand 30 min. with occasional gentle stirring; then trap off into beaker the oil and water layer and any corn debris that may have risen into neck of trap flask. To dissolve the adhering oil, wash out neck of flask with hot alcohol. Add a little more hot H<sub>2</sub>O to flask, stir, let stand 10 min., and trap off again. Set No. 8 or No. 10 sieve 3" in diam. into 400 ml beaker and pour oil and water mixture from trappings onto sieve. Wash corn debris on sieve thoroly with hot alcohol. Filter material that passes thru sieve, washing beaker, sides of funnel, and paper thoroly with hot alcohol to dissolve the oil and speed filtration. Examine paper microscopically.

## GROUND SPICES

*Allspice, Ginger, Cloves, Nutmeg, and Mace*

42.78

## LIGHT AND HEAVY FILTH

Weigh 10 g of sample into 250 ml beaker. Add 150 ml of petroleum benzine and boil gently 15 min. on electric hot plate. Add petroleum benzine occasionally to



keep volume constant. Decant the petroleum benzine onto smooth 7 cm filter paper in Büchner funnel. Add 150 ml of  $\text{CCl}_4$  to beaker and allow to stand with occasional stirring 30 min. Decant spice and  $\text{CCl}_4$  into funnel, leaving heavy residue of sand and soil, if any, in beaker. Add more solvent and repeat if necessary. Transfer residue from beaker to ashless filter and examine for filth. If there is appreciable quantity of residue, place filter paper in tared crucible, ignite, and determine sand and soil. Dry spice in the Büchner funnel thoroly, scrape fine material from paper, and transfer to liter Wildman trap flask, 42.1(a). Add ca 150 ml of  $\text{H}_2\text{O}$ , heat to boiling, and simmer 5 min., with stirring; then cool to room temp. Add 25 ml of gasoline, mix thoroly, and allow to stand 5 min.; then fill flask with  $\text{H}_2\text{O}$  and allow to stand 30 min. Stir every 5 min., trap off, and filter. Add to flask ca 15 ml of gasoline and mix thoroly; trap off and filter second time after 15 min. If second extraction yields appreciable amount of filth, decant most of liquid from flask, add 15 ml of gasoline, and make third extraction. Examine filter papers microscopically.

### *Cinnamon*

#### 42.79

#### HEAVY FILTH AND SAND

Weigh 2 g of sample into 50 ml centrifuge tube and add ca 45 ml of  $\text{CCl}_4$ . Centrifuge 5 min. at 800 r.p.m. in International Size 1 Type SB Centrifuge, using No. 240 head with arm length of 5.25" or its equivalent. Stir layer at top of liquid and repeat centrifuging. Decant ca  $\frac{2}{3}$  of liquid and add fresh  $\text{CCl}_4$  up to 45 ml. Mix thoroly and again centrifuge. Decant as much of liquid as possible without disturbing residue in centrifuge tube. Wash residue onto 11 cm ashless filter with  $\text{CCl}_4$ . Examine under low-power microscope for insect excreta, rodent excreta, and other filth. Ignite filter and residue in tared crucible and determine sand and soil.

#### 42.80

#### LIGHT FILTH

Weigh 10 g of the spice into liter Wildman trap flask, 42.1(a). Add 100 ml of  $\text{H}_2\text{O}$ , mix thoroly, heat to boiling, and allow to simmer 5 min. Cool to room temp., add 25 ml of gasoline, mix thoroly, and allow to stand 5 min. Fill flask with  $\text{H}_2\text{O}$  and allow to stand 30 min., stirring every 5 min. Trap off and filter. Add 15 ml of gasoline to flask. Mix thoroly and allow to stand 15 min. Trap off and filter. If second extraction yields appreciable amount of filth, decant most of liquid from flask, add 15 ml of gasoline, and make third extraction. Examine filter papers.

### *Turmeric*

#### 42.81

#### LIGHT FILTH.—See 42.80

### *Onion Powder*

#### 42.82

#### LIGHT AND HEAVY FILTH

Weigh 50 g of well-mixed sample into 250 ml, hooked-lip beaker. Add 200 ml of  $\text{CCl}_4$ , stir thoroly, and allow to stand 30 min., with occasional stirring. Decant plant tissue onto smooth 7 cm filter paper in Büchner funnel. Add 100 ml of  $\text{CCl}_4$  and repeat decanting until practically no plant tissue remains with sand and soil on bottom of beaker. Transfer residue in beaker to ashless filter paper with stream of  $\text{CCl}_4$  from wash bottle and examine for filth. If there is appreciable quantity of residue, place filter paper in tared crucible, ignite, and determine sand and soil.

*Black and White Pepper***42.83****LIGHT AND HEAVY FILTH**

Weigh 10 g of sample into 250 ml beaker. Add 150 ml of  $\text{CCl}_4$  and allow beaker to stand at least 1 hour, with occasional stirring. Transfer pepper to smooth 7 cm filter paper in Büchner funnel by decantation. Examine residue in beaker for heavy filth. Dry pepper on filter, scraping filter paper, and transfer to liter Wildman trap flask, 42.1(a). Add 100 ml of  $\text{H}_2\text{O}$ , wetting all pepper in flask. Allow to soak 5 min., add 25 ml of gasoline, and mix thoroly. Fill flask with  $\text{H}_2\text{O}$  and allow to stand 30 min., stirring every 5 min. Trap off and filter. Add 15 ml of gasoline to flask and mix thoroly. Trap off and filter second time after 15 min. If second extraction yields appreciable amount of filth, decant most of liquid from flask, add 15 ml of gasoline, and make third extraction. Examine filter papers microscopically.

*Capsicums (red and cayenne pepper, chili powder, paprika, etc.)***42.84****LIGHT AND HEAVY FILTH**

Isolate large elements of filth, such as large larvae, adult insects, clumps of webbing, and insect and rodent excreta pellets, by sifting pepper thru No. 10 sieve. Filth is retained on sieve.

Weigh 10 g of pepper into 250 ml beaker and add 150 ml of petroleum benzine. Boil gently 30 min. on electric hot plate, adding petroleum benzine occasionally to keep volume constant. Decant petroleum benzine onto smooth 7 cm filter paper in Büchner funnel. Add 150 ml of  $\text{CHCl}_3\text{-CCl}_4$  mixture (1+1) and allow to stand 30 min., with occasional stirring. Decant pepper and solvent into the funnel, leaving heavy residue of sand and soil, if any, in beaker. Add more solvent and repeat if necessary. Transfer residue from beaker to ashless filter and examine for filth. If there is appreciable quantity of residue, place filter paper in tared crucible, ignite, and determine sand and soil. Dry pepper in Büchner funnel and transfer to 2 liter Wildman trap flask, 42.1(a). Scrape fine material from filter paper. Add 200 ml of saturated  $\text{Na}_2\text{B}_4\text{O}_7$  soln and heat gently to boiling. Stir constantly and to prevent foaming over remove from heat occasionally or direct stream of air into mouth of flask. Boil 5–15 min. and cool to room temp. Add 35 ml of gasoline, mix thoroly, and allow to stand 5 min. Fill flask with  $\text{H}_2\text{O}$  and allow to stand 30 min., with stirring every 5 min. Trap off and filter. Add ca 20 ml of gasoline to flask and mix thoroly. Trap off and filter second time after 15 min. If second extraction yields appreciable amount of filth, decant most of liquid from flask, add 20 ml of gasoline, and make third extraction. Examine filter papers microscopically.

**42.85****ROT (BASED ON MOLD COUNT)**

Weigh out 10 g of thoroly mixed sample of ground capsicum and transfer to Waring Blendor or equivalent mixer. Add 200 ml of 1%  $\text{NaOH}$  soln in 3 or 4 successive portions, stirring mixture after each addition, washing down with final portion any material that may stick to walls of Blendor. Agitate mixture in Blendor 1 min. With rubber policeman rub down into mixture any material sticking to walls of Blendor and repeat blending 2 min. longer. Add 2 or 3 drops of capryl alcohol to break resulting foam. Mix 100 g of this mixture with 50 g of 3% pectin soln, 42.2(b), and count with Howard mold-counting cell as directed under 42.57.

Occasionally a blended mixture will contain particles of seed tissue that make it difficult to obtain Newton rings in preparing slide for mold counting. A clamp

devised for holding cover slip in place to obviate this difficulty consists of metal plate with circular opening, 2.5 cm in diam., in center of plate; 2 clips attached to anterior edge of plate fasten cover slip in position when slide is placed on plate.

### PEPPER SAUCE AND INTERMEDIATE PRODUCTS

#### *Unground Fermented Crushed Peppers*

42.86

#### LIGHT FILTH

Mix in beaker 100 g of the peppers with 100 ml of gasoline. Thin mixture with small amount of H<sub>2</sub>O if necessary for mixing. Pour mixture into cylindrical, white-enameled pan ca 8" tall × 10" in diam., that has been almost filled with H<sub>2</sub>O. Stir gently. Most of pepper particles sink or remain suspended in the H<sub>2</sub>O, while light filth and some debris come to surface with the gasoline. Decant top layer of gasoline and part of water layer (ca 1.5 liters in all) into 2 liter Wildman trap flask, 42.1(a), thru glass funnel. Rinse funnel and fill trap with H<sub>2</sub>O. Stir, and allow to settle 30 min. Trap off and filter.

42.87

#### HEAVY FILTH

Stir material in pan (42.86) gently and allow to settle ca ½ min. Decant pepper skin fragments. Add more H<sub>2</sub>O and repeat operation until amount of seeds and pepper fragments will not seriously interfere with examination of filter paper for heavy filth. Do not try to get paper entirely free from pepper skin and seeds, because filth will also be decanted. Transfer heavy residue to 7 cm filter and examine.

#### *Pepper Sauce*

42.88

#### LIGHT FILTH

Mix 100 g of sauce with 100 ml of H<sub>2</sub>O and 35 ml of gasoline in 2 liter Wildman trap flask, 42.1(a). Fill flask with H<sub>2</sub>O. Stir, and allow to settle 30 min. Trap off and filter.

42.89

#### HEAVY FILTH

Transfer remainder of material in Wildman trap flask to white-enameled pan. Treat by the sedimentation method as directed under 42.87.

### MAYONNAISE AND SALAD DRESSINGS

42.90

#### FILTH

Weigh 200 g of mayonnaise or salad dressing into 800 ml beaker. Stir in 50 ml of H<sub>3</sub>PO<sub>4</sub>, and mix thoroly. Thin with ca 600 ml of H<sub>2</sub>O. Again mix thoroly and filter thru rapid paper with suction. Examine filter paper microscopically.

### PICKLES AND RELISHES

#### *Whole Pickles*

42.91

#### FILTH

Pour entire contents of jar onto No. 8 sieve nested in No. 100 sieve. Wash jar thoroly to remove any filth adhering to sides, and pour washings thru sieves. Wash pickles thoroly with stream of hot H<sub>2</sub>O, turning from time to time. Transfer material on No. 100 sieve directly to ruled filter paper and examine for filth.

*Chopped Pickles and Relish*

42.92

## FILTH

Add 200 ml of H<sub>2</sub>O to 100 g of pickle, boil 15 min., and cool. Place in Wildman trap flask, 42.1(a), add 25 ml of kerosene, and after stirring well, add sufficient H<sub>2</sub>O to fill flask. Allow to stand 30 min., trap off, filter, and examine in usual manner.

## CONDIMENTAL SEEDS

42.93

## RODENT AND INSECT EXCRETA

Prepare liquid with sp. gr. of 1.16–1.19 by mixing CHCl<sub>3</sub> or CCl<sub>4</sub> with alcohol or petroleum benzine. Mix 200 g of the seed with 500–700 ml of the liquid in 1 quart drug percolator arranged as shown in Fig. 76. Allow mixture to stand 30 min., stirring at ca 5 min. intervals. Trap sediment in lower end of percolator with the plunger and remove lower cork so as to deliver all sediment into beaker. Lift upper cork slightly and rinse tube and cork by allowing small amount of liquid to pass. After stirring top layer, make 2 more separations at 5 min. intervals. Transfer contents of beaker to filter paper, drain off liquid, and examine. Separate rodent excreta and insect excreta, air dry, and weigh each separately to nearest mg.

## PREPARED MUSTARD

42.94

## FILTH

Weigh 100 g of sample into 1 liter beaker. Add 500 ml of filtered 1% pancreatin soln, 42.2(c). Add sufficient NaHCO<sub>3</sub> to bring mixture to pH 8 and digest 2 hours at ca 40°. Wash mixture thru No. 100 sieve with hot H<sub>2</sub>O until drainings are clear. Wash residue on sieve with alcohol followed by CHCl<sub>3</sub> and hot H<sub>2</sub>O. Transfer to liter Wildman trap flask, 42.1(a), by washing sieve with 60% alcohol. Add 25 ml of gasoline, mix thoroly, and allow to stand 5 min. Fill flask with H<sub>2</sub>O and allow to stand 30 min. Stir every 5 min. Trap off, filter, and examine filter paper.

## WHOLE SPICES

42.95

## FILTH BY FLOTATION

Crack the whole spice into small pieces in order to facilitate extraction and proceed as directed under 42.78, using 100 g sample.

## TAMARIND PULP

42.96

## LIGHT FILTH

Mix sample thoroly and weigh out 500 g into 1.5 liter beaker. Add hot H<sub>2</sub>O to within 1" of top of beaker and simmer 15–20 min., stirring occasionally to break up mass of material. Pour contents of beaker thru No. 2 sieve, catching filtrate in convenient receptacle. Break up material on sieve and wash thoroly with hot H<sub>2</sub>O to remove all small adhering particles (filth, etc.). Discard material retained on the No. 2 sieve. Pour material passing thru sieve onto No. 100 sieve and wash thoroly with hot H<sub>2</sub>O. Transfer with cold H<sub>2</sub>O to 2 liter Wildman trap flask, 42.1(a), material retained on the No. 100 sieve. Mix in 35 ml of gasoline and allow to stand 5 min. Fill flask with H<sub>2</sub>O and allow to settle 30 min., stirring every 5 min. (Pulp rising in neck of flask may be worked down by stirring gently with rubber stopper.) Trap off gasoline layer and filter thru 10XX bolting cloth. Add to flask ca 20 ml of gasoline and mix thoroly; trap off and filter second time after 15 min. If second extraction yields appreciable amount of filth, decant most of liquid from flask, add 15 ml of gasoline, and make third extraction. Examine filter papers microscopically.

## CLOTH

## URINE STAINS (8)

**42.97** *Preliminary Examination with Ultra-violet Light*

Examine suspected stains in dark room under ultra-violet light. (Dried urine on textiles usually fluoresces with blue-white color, but color will vary somewhat depending upon natural color of textile and type of lamp and filter used.) Run check patches with known types of urine. For microchemical analysis, outline stained area with pencil under the ultra-violet light.

**42.98** *Urease Test for Urea*

Cut out portion of stained area and transfer 1 or 2 threads to 5 ml crucible or beaker. Save balance of cloth for **42.99** and **42.100**. Leach 10 min. in just enough warm  $H_2O$  to cover material. Remove threads and squeeze out as much fluid as possible with pair of clean, flat-tipped forceps. Remove 2 or 3 drops to a microculture slide with deep cylindrical depression. Add small drop of urease mixture (suspension of  $\frac{1}{2}$  of a 25 mg Dunning urease tablet in 0.5–0.7 ml of  $H_2O$ ). Place small drop of 10%  $H_2PtCl_6$  soln on cover slip and invert over the depression, with hanging drop at center of depression opening. (Cover slip may be sealed on with petrolatum if only minute quantities of urea are suspected.) With evolution of  $NH_3$ , brilliant, highly refractive, octahedral crystals of  $(NH_4)_2PtCl_6$  are formed in hanging drop. Time required for the formation of the crystals will vary from few seconds to 30 min., or even longer in some instances, according to conditions. The crystals may be visible to naked eye and are readily detected under microscope at 100 $\times$ . Certain organic compounds that are volatile and water-soluble may yield crystals in the hanging drop, and if the reagent soln is too concentrated  $H_2PtCl_6$  may crystallize. However, crystal habits of these substances should not be confused with  $(NH_4)_2PtCl_6$ . (When presence of urine stains cannot be confirmed by above procedure, sensitivity of test can be increased by adding drop of N/10 NaOH to the urea-urease mixture, digesting suspected material in usual manner and, after allowing digestion to proceed for ca 1 min., adding the NaOH soln and covering at once with the cover slip containing drop of  $H_2PtCl_6$  soln.) If urease test is positive, obtain additional evidence by further check made with following methods based on formation of urea nitrate and xanthidryl urea.

**42.99** *Xanthidryl Test for Urea*

Place portion of stained cloth, ca  $\frac{1}{2}$ " square, in drop of 50% acetic acid on microscope slide, warm gently, and add very small amount of xanthidryl. Almost immediately small characteristic star-like aggregates of gracefully curved needles are formed if urea is present.

**42.100** *Extraction of Urea and Crystallization of Urea Nitrate*

Place portion of stained cloth in test tube and extract successively 3 times for ca 10 min. each with ca 5 ml of boiling alcohol. Decant alcohol into another test tube and carefully evaporate to dryness in water bath. Prolong heating sufficiently to insure removal of all traces of moisture. Re-extract dry residue 3 times with 5 ml of moisture-free acetone; filter combined extracts into test tube and concentrate to 4–5 ml by immersing tube in warm water bath. Evaporate few drops of acetone extract on microscope slide. On drying, characteristic needle-shaped crystals of urea, which can be identified under microscope, separate out. Draw drop of pure, colorless

HNO<sub>3</sub> over few of the crystals and warm gently. On cooling and after the soln has begun to evaporate, urea nitrate crystallizes out in 6-sided or rhombic tiles. As urea nitrate is somewhat soluble in HNO<sub>3</sub>, it may be necessary to evaporate completely. (Identity of urea separated by alcohol-acetone extraction can also be confirmed by urease-H<sub>2</sub>PtCl<sub>6</sub> method described in 42.98.)

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## GENERAL REFERENCE

"Microanalysis of Food and Drug Products," F & D Circ. No. 1 (1944).

## 43. STANDARD SOLUTIONS

### 43.1

#### GENERAL DIRECTIONS

Accurately calibrated equipment, which meets National Bureau of Standards specifications, should be used. Since alkaline and other corrosive solns will dissolve glass, to avoid volume errors such solns should not be stored in calibrated apparatus, and burets used continuously should be recalibrated periodically.

The working temp. of the standard soln should approximate that of its temp. during standardization. If temp. corrections are necessary, sufficient accuracy may be obtained by use of the following table (1):

VOLUME OF STANDARD SOLUTION	CORRECTION IN MILLILITERS AT—												
	6°	8°	10°	12°	14°	16°	18°	20°	22°	24°	26°	28°	30°
ml.													
10	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	-0.00	-0.01	-0.02	-0.02
20	0.03	0.03	0.03	0.02	0.02	0.01	0.01	0.00	-0.01	-0.02	-0.03	-0.03	-0.03
25	0.04	0.03	0.03	0.03	0.02	0.02	0.01	0.00	-0.01	-0.02	-0.03	-0.04	-0.05
30	0.04	0.04	0.04	0.03	0.03	0.02	0.01	0.00	-0.01	-0.02	-0.04	-0.05	-0.07
40	0.06	0.06	0.05	0.04	0.04	0.03	0.01	0.00	-0.02	-0.03	-0.05	-0.07	-0.09
50	0.07	0.07	0.06	0.06	0.05	0.03	0.02	0.00	-0.02	-0.04	-0.06	-0.09	-0.12

#### SODIUM HYDROXIDE

##### *I. With Potassium Acid Phthalate (2)—Official*

### 43.2

#### APPARATUS

The buret and pipet used should be Bureau of Standards calibrated or should be calibrated by the analyst. Automatic burets should have all exits to the air protected from CO<sub>2</sub> contamination by suitable guard tubes containing soda-lime. All containers should be of alkali-resisting glass.

### 43.3

#### REAGENTS

(a) *Carbonate-free water*.—Prepare by one of the following methods: (1) Boil distilled H<sub>2</sub>O 20 min. and cool with soda-lime protection; (2) bubble air, freed from CO<sub>2</sub> by passing thru a tower of soda-lime, thru distilled H<sub>2</sub>O for 12 hours.

(b) *Sodium hydroxide*.—(1 + 1). To one part of NaOH (reagent quality containing less than 5% Na<sub>2</sub>CO<sub>3</sub>) in a flask add one part of distilled H<sub>2</sub>O and swirl until soln is complete. Close with rubber stopper. Set aside until Na<sub>2</sub>CO<sub>3</sub> has settled, leaving a perfectly clear liquid (ca 10 days).

(c) *Potassium acid phthalate*.—U. S. Bureau of Standards Sample Standard for Acidimetry. Dry for 2 hours at 120°. Cool in desiccator containing H<sub>2</sub>SO<sub>4</sub>.

(d) *Phenolphthalein indicator*.—1.0 g in 100 ml of alcohol.

### 43.4

#### PREPARATION OF STANDARD SOLUTION

The following table gives the approximate amounts of NaOH soln (1 + 1) necessary to make 10 liters of standard solns:

<i>Approx. normality</i>	<i>NaOH soln to be diluted to 10 liters (ml)</i>
0.01	5.4
0.02	10.8
0.10	54.0
0.50	270.0
1.0	540.0

Add required amount of NaOH soln (1+1) to 10 liters of CO<sub>2</sub>-free H<sub>2</sub>O. Check normality, which should be slightly high, as directed under 43.5, and adjust to desired concentration by following formula:  $V_1 = V_2 \times N_2 / N_1$ , where  $N_2$  and  $V_2$  represent normality and volume of stock soln, respectively, and  $V_1$ , volume to which stock soln should be diluted to obtain desired normality,  $N_1$ . Determine exact concn of final soln as directed under 43.5.

## 43.5

## STANDARDIZATION

Accurately weigh sufficient dried KHC<sub>8</sub>H<sub>4</sub>O<sub>4</sub> to titrate ca 40 ml and transfer to 300 ml flask that has been swept free from CO<sub>2</sub>. Add 50 ml of cool CO<sub>2</sub>-free H<sub>2</sub>O. Stopper flask and swirl gently until sample is dissolved. Add 3 drops of the phenolphthalein indicator, and titrate with soln that is being standardized.

Calculate normality ( $N$ ) of standard soln by following formula:

$$N = \frac{\text{g of KHC}_8\text{H}_4\text{O}_4}{\text{ml NaOH} \times 204.216/1000}$$

(Normality value is exact only when phenolphthalein is used as indicator.)

## 43.6

## II. With Constant Boiling Hydrochloric Acid (3)—Official

Accurately weigh by means of weight buret sufficient constant boiling HCl, 43.9, to titrate ca 40 ml into Erlenmeyer flask that has been previously swept free from CO<sub>2</sub>. Add ca 40 ml of CO<sub>2</sub>-free H<sub>2</sub>O, then 3–5 drops of desired indicator, and titrate with soln that is being standardized.

Calculate normality from following equation:

$$N = \frac{\text{g of HCl}}{\text{ml titer} \times \frac{G}{1000}}, \text{ where } G \text{ has the value given in 43.9.}$$

## HYDROCHLORIC ACID

## I. With Standard Sodium Hydroxide (2)—Official, First Action

## 43.7

## PREPARATION OF STANDARD SOLUTIONS

The following table gives approximate amounts of HCl (reagent quality, 35–37% HCl) necessary to make 10 liters of standard solns:

<i>Approx. normality</i>	<i>HCl to be diluted to 10 liters (ml)</i>
0.01	8.9
0.02	17.8
0.10	89.0
0.50	445.0
1.0	890.0



## 43.8

## STANDARDIZATION

Titrate 40 ml against a standard alkali soln of ca same concentration as the acid being standardized, as directed under 43.5, using phenolphthalein as indicator, 43.3(d). Determine normality by following formula:

$$N = \frac{\text{ml standard alkali} \times \text{normality of alkali}}{\text{ml HCl}}$$

If more concentrated than desired, dilute soln to definite normality value by following formula:

$$V_1 = \frac{V_2 \times N_2}{N_1}, \quad \text{where}$$

$N_2$  and  $V_2$  represent normality and volume of stock soln, respectively, and  $V_1$  represents volume to which stock soln should be diluted to obtain desired normality,  $N_1$ .

Check exact concentration of final soln by titration as directed above. Normality will be exact only if same indicator is used in a determination as in the standardization.

If the standard acid soln is to be used with methyl orange as indicator, determine correction for volume of acid required to pass from end point of phenolphthalein to that of methyl orange. Add (4) 3 drops of 1% phenolphthalein soln to 100 ml of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ , and then add sufficient alkali soln to give end point with phenolphthalein. Disregard quantity of alkali soln added and take buret readings from this point. Add 3 drops of 0.02% methyl orange soln and sufficient 0.1  $N$  acid to produce pink color of methyl orange. Titrate back with 0.1  $N$  alkali soln to same end point taken in usual titration (preferably pH 4.2). Buffered solns of 3.8, 4.0, and 4.2 pH are useful in accurately determining methyl orange end point. They may be prepared as follows (4):

pH = 3.8, 2.041 g KH phthalate + 5.30 ml 0.1  $N$  HCl. Dilute to 200 ml.

pH = 4.0, 2.041 g KH phthalate + 0.80 ml 0.1  $N$  NaOH. Dilute to 200 ml.

pH = 4.2, 2.041 g KH phthalate + 7.30 ml 0.1  $N$  NaOH. Dilute to 200 ml.

If the acid and alkali solns are equivalent, quantity of acid—quantity of alkali soln = quantity of acid required to pass from phenolphthalein end point to that of methyl orange.

## 43.9

*II. Constant Boiling Method (5)—Official*

Dilute analytical-reagent grade HCl (35–37% HCl) with equal quantity of  $\text{H}_2\text{O}$ . Adjust sp. gr., if necessary, to 1.10 (spindle). Place 1500 ml in a 2 liter flask connected to a straight inner-tube condenser, using rubber or ground-glass stoppers. (Distilling system should be of resistant glass constructed so as completely to condense all distillate and at same time maintain exact atmospheric pressure thruout.) To prevent superheating of any part of the system, place ten 1 inch lengths of small-diameter, resistant glass tubing in the boiling flask and so encase flask in sheet asbestos that hot gases from the flame strike flask only at bottom. Distil continuously at rate of 5–10 ml per min. When 1125 ml has been distilled, change receivers and catch the next 225 ml, which is the constant boiling mixture, in Erlenmeyer flask, with end of condenser inserted into flask but not below surface of liquid. Read barometer to nearest mm at beginning and end of collection of the 225 ml portion and average readings.

Calculate air weight (in g) of this constant boiling HCl ( $G$ ) required to give one equivalent weight of HCl from following equation:

$$G = \frac{P + 7680}{46.8386},$$

where  $P$  is barometric pressure in mm of Hg, corrected to 0°. (Formula is applicable to pressures of 540–780 mm Hg.)

Weigh out required quantity of constant boiling HCl in tared beaker or flask to an accuracy of at least 1 part in 10,000 to make any quantity of any exact concentration. Dilute immediately and finally make up to volume with CO<sub>2</sub>-free H<sub>2</sub>O at desired temp.

### III. With Borax (6)—Official

#### 43.10

#### REAGENTS

(a) *Methyl red indicator*.—Dissolve 100 mg of methyl red in 60 ml of alcohol and dilute with H<sub>2</sub>O to 100 ml.

(b) *Sodium borate*.—U.S.P. quality or better; soln of 5 g of the salt in 95 ml of warm H<sub>2</sub>O should pass following purity test:

- (1) *Insoluble impurities*.—Should be clear and colorless.
- (2) *Chloride*.—20 ml must not give an opalescence with HNO<sub>3</sub> and AgNO<sub>3</sub> that is stronger than that given by 20 ml of a soln containing 5 mg of Cl/liter.
- (3) *Sulfate*.—20 ml should give no precipitate with acetic acid and BaCl<sub>2</sub> after standing 30 min.
- (4) *Calcium*.—20 ml of hot soln should give no turbidity with NH<sub>4</sub> oxalate after cooling.
- (5) *Magnesium*.—20 ml of soln must not give any microcrystalline precipitate with ammonia and phosphate after standing 24 hours.

(c) *Reference soln*.—Prepare a reference soln of H<sub>3</sub>BO<sub>3</sub>, NaCl, and indicator corresponding to composition and volume of the soln at equivalence point. For use in determination of end point of a titration with 0.1  $N$  acid, reference soln should be 0.1  $M$  in H<sub>3</sub>BO<sub>3</sub>, and 0.05  $M$  in NaCl.

(d) *Standard borax*.—Saturate 300 ml of H<sub>2</sub>O at 55° (not higher) with Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> · 10H<sub>2</sub>O (ca 45 g). Filter at this temp. thru folded filter into 500 ml Erlenmeyer flask. Cool filtrate to ca 10°, with continuous agitation during crystallization. Decant supernatant liquid. Rinse precipitate once with 25 ml of cold H<sub>2</sub>O. Dissolve crystals in just enough H<sub>2</sub>O at temp. of 55° to insure complete soln (ca 200 ml). Recrystallize by cooling to ca 10°, agitating flask during crystallization. Filter crystals onto small Büchner funnel with suction. Wash precipitate once with 25 ml of ice-cold H<sub>2</sub>O. Dry crystals (7) by washing with two 20 ml portions of alcohol, drying after each washing with suction. Follow with two successive 20 ml portions of ether. (Just prior to use free the alcohol and ether from any possible reacting acids by shaking each vigorously with 2 or 3 g of the pure, dry Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> · 10H<sub>2</sub>O, then filtering.) After spreading on a watch-glass, immediately place the dried Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> in desiccator over soln saturated with respect to both sugar and NaCl and allow to remain at least 24 hours before using. Then transfer the pure Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> · 10H<sub>2</sub>O to container that has ground-glass stopper and store in desiccator when not in use (stable under these conditions for 1 year).

## 43.11

## STANDARDIZATION

Accurately weigh sufficient standard  $\text{Na}_2\text{B}_4\text{O}_7 \cdot \text{H}_2\text{O}$  to titrate ca 40 ml and transfer to 300 ml flask. Add 40 ml of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  and stopper flask. Swirl gently until sample is in soln. Add 4 drops of methyl red indicator and titrate with soln that is being standardized to equivalence point as indicated by reference soln. Calculate normality ( $N$ ) of the standard soln by following formula:

$$N = \frac{\text{g of Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}}{\text{ml of acid} \times 190.72/1000}$$

## IV. With Sodium Carbonate (6)—Official

## 43.12

## REAGENTS

(a) *Methyl orange indicator*.—0.1% in  $\text{H}_2\text{O}$ .

(b) *Sodium bicarbonate*.—C.P. Should pass following tests for purity:

- (1) *Chloride*.—0.5 g of  $\text{NaHCO}_3$  dissolved in 10 ml of 2  $N$   $\text{HNO}_3$  (free of Cl) must give no opalescence with  $\text{AgNO}_3$ .
- (2) *Sulfate*.—0.5 g of  $\text{NaHCO}_3$  in 10 ml of 2  $N$  acetic acid should give no turbidity or separation of  $\text{BaSO}_4$  after addition of  $\text{BaCl}_2$  and standing 15 min.

(c) *Reference soln*.—80 ml of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$  with 3 or 4 drops of methyl orange indicator.

(d) *Anhydrous sodium carbonate (8)*.—Heat 250 ml of  $\text{H}_2\text{O}$  to  $80^\circ$  and add  $\text{NaHCO}_3$ , stirring until no more dissolves. Then filter soln thru folded filter (use of hot water funnel is desirable) into Erlenmeyer flask. Cool filtrate to ca  $10^\circ$  with constant swirling during crystallization. The fine crystalline trona and bicarbonate that separates out has the approximate composition:  $\text{Na}_2\text{CO}_3 \cdot \text{NaHCO}_3 \cdot 2\text{H}_2\text{O}$ . Pour off mother liquor. Drain crystals by suction and wash once with cold  $\text{H}_2\text{O}$ .

Transfer precipitate, being careful not to include any fibers of filter paper, to large flat-bottomed dish. Heat in electric oven or furnace with pyrometer control at temp. of  $290^\circ$  for 1 hour. Stir contents occasionally with Pt wire. After heating, cool Pt dish and contents in desiccator. Store the anhydrous  $\text{Na}_2\text{CO}_3$  in container having ground-glass stopper in desiccator containing a good desiccant. Dry the salt at  $120^\circ$  just before using.

## 43.13

## STANDARDIZATION

Accurately weigh sufficient anhydrous  $\text{Na}_2\text{CO}_3$  to titrate ca 40 ml and transfer to 300 ml Erlenmeyer flask. Add 40 ml of  $\text{H}_2\text{O}$  to dissolve the salt. Add 3 drops of the methyl orange indicator and titrate (9) until color begins to deviate from the  $\text{H}_2\text{O}$  tint (reference soln). (Equivalence point has not been reached.) Boil the soln gently 2 min., then cool. Titrate until color is barely different from the  $\text{H}_2\text{O}$  tint (of the indicator).

Calculate the normality ( $N$ ) of standard soln by following formula:

$$N = \frac{\text{g of Na}_2\text{CO}_3}{\text{ml of acid} \times 53.002/1000}$$

## SULFURIC ACID

*I. Standard Borax Method (8)—Tentative*

## 43.14

## PREPARATION OF STANDARD SOLUTION

The following table gives approximate amounts of  $\text{H}_2\text{SO}_4$  (reagent quality ca 94%  $\text{H}_2\text{SO}_4$ ) necessary to make 10 liters of standard solns:

<i>Approx. normality</i>	<i><math>\text{H}_2\text{SO}_4</math> to be diluted to 10 liters (ml)</i>
0.01	2.8
0.02	5.7
0.10	28.4
0.50	141.8
1.0	283.5

## 43.15

STANDARDIZATION.—See 43.11.

## 43.16

*II. Specific Gravity Method (10)—Official*

Dilute analytical-reagent quality, ca 94%  $\text{H}_2\text{SO}_4$ , with sufficient  $\text{H}_2\text{O}$  to make convenient quantity of ca 70%  $\text{H}_2\text{SO}_4$  soln. Determine sp. gr. in air at convenient temp. (0–40°) as directed in 16.4 (or sp. gr. may be determined by means of Sprengel pycnometer), protecting soln from contact with the air. Calculate exact percentage of  $\text{H}_2\text{SO}_4$  by means of the equation:  $P = S(85.87 + 0.05T - 0.0004t^2) - 69.82$ ; where  $P$  = percentage of  $\text{H}_2\text{SO}_4$ , by weight; and  $S$  = sp. gr. (in air) at  $T^\circ$ , compared with  $\text{H}_2\text{O}$  at  $t^\circ$ .

Weigh  $W$  grams of the prepared acid containing  $P\%$   $\text{H}_2\text{SO}_4$  and dilute to  $n$  liters to make required exactly standard soln containing  $A$  grams of  $\text{H}_2\text{SO}_4$ /liter.  $W$  may be calculated by the equation:

$$W = nA \times \frac{100}{P}.$$

## POTASSIUM PERMANGANATE (11)—OFFICIAL

## 43.17

## PREPARATION OF STANDARD SOLUTION

Dissolve slightly more than desired equivalent weight (3.2 g for 0.1  $N$ ) of  $\text{KMnO}_4$  (A.R. or C.P. grade) in 1 liter of  $\text{H}_2\text{O}$ . Boil soln 1 hour. Protect from dust and allow to stand overnight. Clean thoroly 15 cm glass funnel, perforated porcelain plate from Caldwell crucible, and glass-stoppered bottle (preferably of brown glass), with warm  $\text{H}_2\text{SO}_4$ - $\text{K}_2\text{Cr}_2\text{O}_7$  soln. Digest asbestos for use in Gooch crucibles on steam bath 1 hour, with ca 0.1  $N$   $\text{KMnO}_4$  that has been acidified with a few drops of  $\text{H}_2\text{SO}_4$  (1+3). Allow to settle, decant, and replace with  $\text{H}_2\text{O}$ . Prepare glass funnel by placing porcelain plate in apex, make pad of asbestos ca 3 mm thick on plate, and wash free from acid. Pad should not be too tightly packed and only moderate suction should be applied. Insert stem of funnel into neck of bottle and filter the  $\text{KMnO}_4$  soln directly into bottle without aid of suction.

## 43.18

## STANDARDIZATION

Weigh 0.25–0.30 g of Bureau of Standards  $\text{Na}_2\text{C}_2\text{O}_4$  in sufficient  $\text{H}_2\text{O}$  to make soln ca 0.1  $N$ . Add 15 ml of 4  $N$   $\text{H}_2\text{SO}_4$  for each 50 ml of soln. Heat to 75–85° and

titrate with the  $\text{KMnO}_4$  soln, maintaining this temp. thruout titration. Add the  $\text{KMnO}_4$  slowly, especially at beginning, and wait each time until soln becomes colorless. Continue titration to end point, with continuous stirring. Correct for excess of  $\text{KMnO}_4$  used for end point by matching color in another beaker containing same quantity of acid and hot  $\text{H}_2\text{O}$ .

Solutions more dilute than 0.1 *N* may be prepared by diluting more concentrated soln with  $\text{H}_2\text{O}$  that has been distilled over alkaline  $\text{KMnO}_4$ , using usual precautions to prevent contamination with organic matter.

#### ARSENIOUS OXIDE (12)—OFFICIAL

##### 43.19

##### REAGENT

*Arsenious oxide soln.*—Use National Bureau of Standards sample. Dry 1 hour at  $105^\circ$  immediately before using.

##### 43.20

##### PREPARATION OF STANDARD SOLUTION

Weigh the  $\text{As}_2\text{O}_3$  accurately by difference from small glass-stoppered weighing bottle (use ca 4.95 g/liter for 0.1 *N*). Dissolve in normal  $\text{NaOH}$  soln (50 ml for each 5 g of  $\text{As}_2\text{O}_3$ ) in flask or beaker by heating on steam bath. Add ca same quantity of normal  $\text{H}_2\text{SO}_4$ . Cool, and transfer mixture quantitatively to volumetric flask and make to volume. (Soln must be neutral to litmus, not alkaline.) Correct for volume changes due to temperature.

#### IODINE (12)—OFFICIAL

##### 43.21

##### PREPARATION OF STANDARD SOLUTION

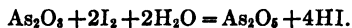
Dissolve weighed quantity of I (12.7 g/liter for 0.1 *N* soln), and KI in proportion of 20 g/13 g of I, in 50 ml of  $\text{H}_2\text{O}$ . When I has dissolved, transfer soln to glass-stoppered graduated flask. Dilute to mark with  $\text{H}_2\text{O}$  and mix thoroly. Keep soln in dark brown glass-stoppered bottle away from light and restandardize as frequently as necessary.

##### 43.22

##### STANDARDIZATION

Transfer accurately measured portion of standard  $\text{As}_2\text{O}_3$  soln, 43.20, (40–50 ml of ca 0.1 *N* soln for 0.1 *N* I soln) into Erlenmeyer flask. Make slightly acid with  $\text{H}_2\text{SO}_4$  (1+10), neutralize with solid  $\text{NaHCO}_3$ , and add ca 2 g in excess. Titrate with the I soln, using ca 0.2% starch soln (5 ml for each 100 ml) as indicator. Saturate the soln with  $\text{CO}_2$  at end of titration by adding 1 ml of the dilute  $\text{H}_2\text{SO}_4$  just before end point is reached.

From quantities of I and  $\text{As}_2\text{O}_3$  solns used calculate titer of I soln on basis of following equation:



#### SILVER NITRATE (13)—OFFICIAL

##### 43.23

##### PREPARATION OF STANDARD SOLUTION

Dissolve slightly more than theoretical quantity of  $\text{AgNO}_3$  (equivalent weight, 169.89) in halogen-free  $\text{H}_2\text{O}$  and dilute to volume. Have all glassware thoroly clean, avoid contact with dust, and keep prepared soln in amber, glass-stoppered bottles and away from light.

*I. Mohr Method*

## 43.24

## REAGENTS

(a) *Potassium chloride*.—Recrystallize KCl 3 times with H<sub>2</sub>O, dry at 110°, and then heat at ca 500° to constant weight. Equivalent wt. of KCl = 74.553.

(b) *Potassium chromate soln.*—5% soln of K<sub>2</sub>CrO<sub>4</sub> in H<sub>2</sub>O.

## 43.25

## STANDARDIZATION

Weigh accurately sufficient quantity of KCl to yield titration of ca 40 ml (ca 0.3 g for 0.1 *N* soln) and transfer to 250 ml glass-stoppered Erlenmeyer flask with 40 ml of H<sub>2</sub>O. Add 1 ml of the K<sub>2</sub>CrO<sub>4</sub> soln and titrate with the AgNO<sub>3</sub> soln until the appearance of first perceptible pale red-brown color. Subtract from titration the quantity of AgNO<sub>3</sub> soln required to produce the end-point color in 75 ml of H<sub>2</sub>O containing 1 ml of the K<sub>2</sub>CrO<sub>4</sub> soln. Calculate normality of the AgNO<sub>3</sub> soln.

*II. Volhard Method*

## 43.26

## REAGENTS

(a) *Ferric alum soln.*—Saturated soln of FeNH<sub>4</sub>(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O in H<sub>2</sub>O.

(b) *Nitric acid.*—(1 + 1).

(c) *Nitric acid.*—2%.

(d) *Potassium or ammonium thiocyanate standard soln.*—Prepare ca 0.1 *N* soln from reagent that shows no chloride. Determine working titer by accurately measuring 40–50 ml of the standard AgNO<sub>3</sub> soln, adding 2 ml of the Fe alum soln and 5 ml of the (1 + 1) HNO<sub>3</sub> soln, and titrating with the thiocyanate soln until soln shows pale rose color after vigorous shaking.

## 43.27

## STANDARDIZATION

Weigh accurately sufficient quantity of the KCl, 43.24(a), to yield titration of ca 40 ml (ca 0.3 g for 0.1 *N* soln) and transfer to 250 ml glass-stoppered Erlenmeyer flask with 40 ml of H<sub>2</sub>O. Add 5 ml of the (1 + 1) HNO<sub>3</sub> soln and run in excess of the AgNO<sub>3</sub> soln. Mix, and allow to stand a few minutes protected from light. Filter thru Gooch crucible prepared with medium pad of asbestos previously rinsed with the 2% HNO<sub>3</sub>. Wash flask and precipitate with several small portions of the 2% HNO<sub>3</sub>, passing washings thru crucible until filtrate and washings measure ca 150 ml. Add 2 ml of the Fe alum soln and titrate residual AgNO<sub>3</sub> with the thiocyanate soln. From the titration, together with ratio of the two standard solns, calculate normality of the AgNO<sub>3</sub> soln. (Errors of a blank are compensating and may be disregarded.) Equivalent weight of KCl = 74.55.

## SODIUM THIOSULFATE (14)—OFFICIAL, FIRST ACTION

## 43.28

## PREPARATION OF STANDARD SOLUTION

Dissolve ca 25 g of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O in 1 liter of H<sub>2</sub>O. Boil gently for 5 min. and transfer while hot to storage bottle that has been cleaned with hot H<sub>2</sub>SO<sub>4</sub>-K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> soln and rinsed with warm boiled H<sub>2</sub>O. (It is advisable to temper bottle, if not made of resistant glass, before adding hot soln.) Store soln in dark, cool place, and do not return unused portions to stock bottle. If solns less concentrated than 0.1 *N* are desired, prepare by dilution with boiled H<sub>2</sub>O. (More dilute solns are less stable and should be prepared just before use.)

## 43.29

## STANDARDIZATION

Dissolve 0.20–0.23 g of  $K_2Cr_2O_7$  (thrice recrystallized and dried at  $200^\circ$ ) in a soln of 2 g of KI in 20 ml of  $N$  HCl and 80 ml of  $H_2O$ , contained in glass-stoppered flask. Allow mixture to stand 10 min. in dark, then titrate with the  $Na_2S_2O_3$  soln, 43.28, adding starch soln after most of the I has been consumed.

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*International atomic weights.<sup>1</sup>*  
1943

44.1

	SYMBOL	ATOMIC NUMBER	ATOMIC WEIGHT		SYMBOL	ATOMIC NUMBER	ATOMIC WEIGHT
Aluminum.....	Al	13	26.97	Molybdenum...	Mo	42	95.95
Antimony.....	Sb	51	121.76	Neodymium....	Nd	60	144.27
Argon.....	A	18	39.944	Neon.....	Ne	10	20.183
Arsenic.....	As	33	74.91	Nickel.....	Ni	28	58.69
Barium.....	Ba	56	137.36	Nitrogen.....	N	7	14.008
Beryllium.....	Be	4	9.02	Osmium.....	Os	76	190.2
Bismuth.....	Bi	83	209.00	Oxygen.....	O	8	16.0000
Boron.....	B	5	10.82	Palladium.....	Pd	46	106.7
Bromine.....	Br	35	79.916	Phosphorus....	P	15	30.98
Cadmium.....	Cd	48	112.41	Platinum.....	Pt	78	195.23
Calcium.....	Ca	20	40.08	Potassium.....	K	19	39.096
Carbon.....	C	6	12.010	Praseodymium..	Pr	59	140.92
Cerium.....	Ce	58	140.13	Protactinium...	Pa	91	231
Cesium.....	Cs	55	132.91	Radium.....	Ra	88	226.05
Chlorine.....	Cl	17	35.457	Radon.....	Rn	86	222
Chromium.....	Cr	24	52.01	Rhenium.....	Re	75	186.31
Cobalt.....	Co	27	58.94	Rhodium.....	Rh	45	102.91
Columbium.....	Cb	41	92.91	Rubidium.....	Rb	37	85.48
Copper.....	Cu	29	63.57	Ruthenium.....	Ru	44	101.7
Dysprosium....	Dy	66	162.46	Samarium.....	Sm	62	150.43
Erbium.....	Er	68	167.2	Scandium.....	Sc	21	45.10
Europium.....	Eu	63	152.0	Selenium.....	Se	34	78.96
Fluorine.....	F	9	19.00	Silicon.....	Si	14	28.06
Gadolinium....	Gd	64	156.9	Silver.....	Ag	47	107.880
Gallium.....	Ga	31	69.72	Sodium.....	Na	11	22.997
Germanium....	Ge	32	72.60	Strontium.....	Sr	38	87.63
Gold.....	Au	79	197.2	Sulfur.....	S	16	32.06
Hafnium.....	Hf	72	178.6	Tantalum.....	Ta	73	180.88
Helium.....	He	2	4.003	Tellurium.....	Te	52	127.61
Holmium.....	Ho	67	164.94	Terbium.....	Tb	65	159.2
Hydrogen.....	H	1	1.0080	Thallium.....	Tl	81	204.39
Indium.....	In	49	114.76	Thorium.....	Th	90	232.12
Iodine.....	I	53	126.92	Thulium.....	Tm	69	169.4
Iridium.....	Ir	77	193.1	Tin.....	Sn	50	118.70
Iron.....	Fe	26	55.85	Titanium.....	Ti	22	47.90
Krypton.....	Kr	36	83.7	Tungsten.....	W	74	183.92
Lanthanum....	La	57	138.92	Uranium.....	U	92	238.07
Lead.....	Pb	82	207.21	Vanadium.....	V	23	50.95
Lithium.....	Li	3	6.940	Xenon.....	Xe	54	131.3
Lutecium.....	Lu	71	174.99	Ytterbium.....	Yb	70	173.04
Magnesium....	Mg	12	24.32	Yttrium.....	Y	39	88.92
Manganese.....	Mn	25	54.93	Zinc.....	Zn	30	65.38
Mercury.....	Hg	80	200.61	Zirconium.....	Zr	40	91.22

<sup>1</sup> Taken from *J. Am. Chem. Soc.*, 65, 1446 (1943).

**44.2 Various strength solutions of the common acids, alkalies, and alcohol.<sup>1</sup>**

(a) *Hydrochloric Acid Solns*: Specification requires not less than 35% HCl by weight. Sp. gr. = 1.1778 at 15°. Mix with water and make up to 1 liter.

HCl STRENGTH DESIRED	HYDROCHLORIC ACID REQUIRED		
	GRAMS	ML	
5	14.29	12.13	Normal solution
10	28.57	24.26	
15	42.85	36.39	
20	57.14	48.52	
36.46	104.17	88.45	
50	142.86	121.29	
100	285.71	242.58	
150	428.57	363.88	Constant boiling Sp. gr. 1.125
200	571.43	485.17	
222.6	636.00	539.99	
278.4	795.43	675.35	
300	857.14	727.75	

(b) *Sulfuric Acid Solns*: Specification requires not less than 94% H<sub>2</sub>SO<sub>4</sub> by weight. Sp. gr. = 1.835 at 15°. Pour acid into excess of water and make up to 1 liter.

H <sub>2</sub> SO <sub>4</sub> STRENGTH DESIRED	SULFURIC ACID REQUIRED		
	GRAMS	ML	
5	5.32	3.0	For crude fiber
12.5	13.29	7.2	
20	21.28	11.6	
30	31.91	17.4	
40	42.55	23.2	Normal solution
49	52.13	28.4	
100	106.38	58.0	
150	159.57	87.0	
250	265.96	144.9	
300	319.15	173.9	
400	425.53	231.9	

(c) *Nitric Acid Solns*: Specification requires not less than 68% HNO<sub>3</sub> by weight. Sp. gr. = 1.4146 at 15°. 1 ml of concentrated HNO<sub>3</sub> contains ca 0.96 g of HNO<sub>3</sub>. Mix with water and make up to 1 liter.

HNO <sub>3</sub> STRENGTH DESIRED	NITRIC ACID REQUIRED	
	GRAMS	ML
5	7.35	5.2
10	14.71	10.4
20	29.41	20.8
30	44.12	31.2
40	58.82	41.6
50	73.53	52.0
63	92.65	65.5
70	102.94	72.8
100	147.06	104.0
150	220.59	156.0
200	294.12	207.9
300	441.18	312.9

<sup>1</sup> Prepared by G. C. Spencer and H. J. Fisher.

*Various strength solutions of common acids, alkalies, and alcohol.—Concluded.*

44.2

(d) *Ammonia Solns*: Specification requires not less than 27%  $\text{NH}_3$  by weight. Sp. gr. = 0.9. Mix and make to 1 liter.

NH <sub>3</sub> STRENGTH DESIRED	REAGENT AMMONIA REQUIRED	
	GRAMS	ML
5	18.52	20.6
10	37.04	41.1
15	55.55	61.7
20	74.07	82.3
25	92.59	102.9
50	185.18	205.8
75	277.77	308.6
100	370.37	411.5
150	555.55	617.3
200	740.74	823.0

(e) *Sodium Hydroxide Solns*: Specification requires 95% of NaOH in sticks of caustic soda. Dissolve and dilute to 1 liter.

NaOH STRENGTH DESIRED	SODIUM HYDROXIDE REQUIRED	
	GRAMS	
12.5	13.16	For crude fiber Normal solution
30	31.58	
40	42.11	
50	52.63	
75	78.95	
100	105.26	
150	157.89	
200	210.53	
250	263.16	
300	315.79	

(f) *Alcoholic Solns*<sup>1</sup>: Specification requires 95%  $\text{C}_2\text{H}_5\text{OH}$  by volume. Sp. gr. = 0.810 at 25°. Mix and make to 1 liter.

ALCOHOL STRENGTH DESIRED	ALCOHOL REQUIRED	
	GRAMS	ML
50	42.63	52.6
100	85.26	105.3
150	127.89	157.9
200	170.52	210.5
250	213.16	263.2
300	255.78	315.9
400	341.04	421.1
500	426.32 (proof)	526.3
700	596.84	736.8

<sup>1</sup> Alcohol of any desired strength may be obtained by taking the number of ml of 95% alcohol equivalent to the desired strength and making the soln up to 95 ml. For example: To obtain a soln of 70% alcohol, take 70 ml of 95% alcohol and dilute to 95 ml.

44.3 *Degrees Brix, specific gravity, and degrees Baumé of sugar solutions<sup>1</sup>  
(Plato's Table<sup>2</sup>).*

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
0.0	1.00000	0.998234	0.00	9.0	1.03586	1.034029	5.02
0.2	1.00078	0.999010	0.11	9.2	1.03668	1.034850	5.13
0.4	1.00155	0.999786	0.22	9.4	1.03750	1.035671	5.24
0.6	1.00233	1.000563	0.34	9.6	1.03833	1.036494	5.35
0.8	1.00311	1.001342	0.45	9.8	1.03915	1.037318	5.46
1.0	1.00389	1.002120	0.56	10.0	1.03998	1.038143	5.57
1.2	1.00467	1.002897	0.67	10.2	1.04081	1.038970	5.68
1.4	1.00545	1.003675	0.79	10.4	1.04164	1.039797	5.80
1.6	1.00623	1.004453	0.90	10.6	1.04247	1.040626	5.91
1.8	1.00701	1.005234	1.01	10.8	1.04330	1.041456	6.02
2.0	1.00779	1.006015	1.12	11.0	1.04413	1.042288	6.13
2.2	1.00858	1.006796	1.23	11.2	1.04497	1.043121	6.24
2.4	1.00936	1.007580	1.34	11.4	1.04580	1.043954	6.35
2.6	1.01015	1.008363	1.46	11.6	1.04664	1.044788	6.46
2.8	1.01093	1.009148	1.57	11.8	1.04747	1.045625	6.57
3.0	1.01172	1.009934	1.68	12.0	1.04831	1.046462	6.68
3.2	1.01251	1.010721	1.79	12.2	1.04915	1.047300	6.79
3.4	1.01330	1.011510	1.90	12.4	1.04999	1.048140	6.90
3.6	1.01409	1.012298	2.02	12.6	1.05084	1.048980	7.02
3.8	1.01488	1.013089	2.13	12.8	1.05168	1.049822	7.13
4.0	1.01567	1.013881	2.24	13.0	1.05252	1.050665	7.24
4.2	1.01647	1.014673	2.35	13.2	1.05337	1.051510	7.35
4.4	1.01726	1.015467	2.46	13.4	1.05422	1.052356	7.46
4.6	1.01806	1.016261	2.57	13.6	1.05506	1.053202	7.57
4.8	1.01886	1.017058	2.68	13.8	1.05591	1.054050	7.68
5.0	1.01965	1.017854	2.79	14.0	1.05677	1.054900	7.79
5.2	1.02045	1.018652	2.91	14.2	1.05762	1.055751	7.90
5.4	1.02125	1.019451	3.02	14.4	1.05847	1.056602	8.01
5.6	1.02206	1.020251	3.13	14.6	1.05933	1.057455	8.12
5.8	1.02286	1.021053	3.24	14.8	1.06018	1.058310	8.23
6.0	1.02366	1.021855	3.35	15.0	1.06104	1.059165	8.34
6.2	1.02447	1.022659	3.46	15.2	1.06190	1.060022	8.45
6.4	1.02527	1.023463	3.57	15.4	1.06276	1.060880	8.56
6.6	1.02608	1.024270	3.69	15.6	1.06362	1.061738	8.67
6.8	1.02689	1.025077	3.80	15.8	1.06448	1.062598	8.78
7.0	1.02770	1.025885	3.91	16.0	1.06534	1.063460	8.89
7.2	1.02851	1.026694	4.02	16.2	1.06621	1.064324	9.00
7.4	1.02932	1.027504	4.13	16.4	1.06707	1.065188	9.11
7.6	1.03013	1.028316	4.24	16.6	1.06794	1.066054	9.22
7.8	1.03095	1.029128	4.35	16.8	1.06881	1.066921	9.33
8.0	1.03176	1.029942	4.46	17.0	1.06968	1.067789	9.45
8.2	1.03258	1.030757	4.58	17.2	1.07055	1.068658	9.56
8.4	1.03340	1.031573	4.69	17.4	1.07142	1.069529	9.67
8.6	1.03422	1.032391	4.80	17.6	1.07229	1.070400	9.78
8.8	1.03504	1.033209	4.91	17.8	1.07317	1.071273	9.89

<sup>1</sup> Bur. Standards Circ. C440, 1942, pp. 614, 626.

<sup>2</sup> Based upon figures prepared by the Kaiserliche Normal-Eichungs-Kommission and accepted by the International Commission for Uniform Methods of Sugar Analysis.

*Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.—Continued.*

44.3

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
18.0	1.07404	1.072147	10.00	27.0	1.11480	1.112828	14.93
18.2	1.07492	1.073023	10.11	27.2	1.11573	1.113763	15.04
18.4	1.07580	1.073900	10.22	27.4	1.11667	1.114697	15.15
18.6	1.07668	1.074777	10.33	27.6	1.11761	1.115635	15.26
18.8	1.07756	1.075657	10.44	27.8	1.11855	1.116572	15.37
19.0	1.07844	1.076537	10.55	28.0	1.11949	1.117512	15.48
19.2	1.07932	1.077419	10.66	28.2	1.12043	1.118453	15.59
19.4	1.08021	1.078302	10.77	28.4	1.12138	1.119395	15.69
19.6	1.08110	1.079187	10.88	28.6	1.12232	1.120339	15.80
19.8	1.08198	1.080072	10.99	28.8	1.12327	1.121284	15.91
20.0	1.08287	1.080959	11.10	29.0	1.12422	1.122231	16.02
20.2	1.08376	1.081848	11.21	29.2	1.12517	1.123179	16.13
20.4	1.08465	1.082737	11.32	29.4	1.12612	1.124128	16.24
20.6	1.08554	1.083628	11.43	29.6	1.12707	1.125079	16.35
20.8	1.08644	1.084520	11.54	29.8	1.12802	1.126030	16.46
21.0	1.08733	1.085414	11.65	30.0	1.12898	1.126984	16.57
21.2	1.08823	1.086309	11.76	30.2	1.12993	1.127939	16.67
21.4	1.08913	1.087205	11.87	30.4	1.13089	1.128896	16.78
21.6	1.09003	1.088101	11.98	30.6	1.13185	1.129853	16.89
21.8	1.09093	1.089000	12.09	30.8	1.13281	1.130812	17.00
22.0	1.09183	1.089900	12.20	31.0	1.13378	1.131773	17.11
22.2	1.09273	1.090802	12.31	31.2	1.13474	1.132735	17.22
22.4	1.09364	1.091704	12.42	31.4	1.13570	1.133698	17.33
22.6	1.09454	1.092607	12.52	31.6	1.13667	1.134663	17.43
22.8	1.09545	1.093513	12.63	31.8	1.13764	1.135628	17.54
23.0	1.09636	1.094420	12.74	32.0	1.13861	1.136596	17.65
23.2	1.09727	1.095328	12.85	32.2	1.13958	1.137565	17.76
23.4	1.09818	1.096236	12.96	32.4	1.14055	1.138534	17.87
23.6	1.09909	1.097147	13.07	32.6	1.14152	1.139506	17.98
23.8	1.10000	1.098058	13.18	32.8	1.14250	1.140479	18.08
24.0	1.10092	1.098971	13.29	33.0	1.14347	1.141453	18.19
24.2	1.10183	1.099886	13.40	33.2	1.14445	1.142429	18.30
24.4	1.10275	1.100802	13.51	33.4	1.14543	1.143405	18.41
24.6	1.10367	1.101718	13.62	33.6	1.14641	1.144384	18.52
24.8	1.10459	1.102637	13.73	33.8	1.14739	1.145363	18.63
25.0	1.10551	1.103557	13.84	34.0	1.14837	1.146345	18.73
25.2	1.10643	1.104478	13.95	34.2	1.14936	1.147328	18.84
25.4	1.10736	1.105400	14.06	34.4	1.15034	1.148313	18.95
25.6	1.10828	1.106324	14.17	34.6	1.15133	1.149298	19.06
25.8	1.10921	1.107248	14.28	34.8	1.15232	1.150286	19.17
26.0	1.11014	1.108175	14.39	35.0	1.15331	1.151275	19.28
26.2	1.11106	1.109103	14.49	35.2	1.15430	1.152265	19.38
26.4	1.11200	1.110033	14.60	35.4	1.15530	1.153256	19.49
26.6	1.11293	1.110963	14.71	35.6	1.15629	1.154249	19.60
26.8	1.11386	1.111895	14.82	35.8	1.15729	1.155242	19.71

## 44.3

*Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.—Continued.*

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
36.0	1.15828	1.156238	19.81	45.0	1.20467	1.202540	24.63
36.2	1.15928	1.157235	19.92	45.2	1.20573	1.203603	24.74
36.4	1.16028	1.158233	20.03	45.4	1.20680	1.204668	24.85
36.6	1.16128	1.159233	20.14	45.6	1.20787	1.205733	24.95
36.8	1.16228	1.160233	20.25	45.8	1.20894	1.206801	25.06
37.0	1.16329	1.161236	20.35	46.0	1.21001	1.207870	25.17
37.2	1.16430	1.162240	20.46	46.2	1.21108	1.208940	25.27
37.4	1.16530	1.163245	20.57	46.4	1.21215	1.210013	25.38
37.6	1.16631	1.164252	20.68	46.6	1.21323	1.211086	25.48
37.8	1.16732	1.165259	20.78	46.8	1.21431	1.212162	25.59
38.0	1.16833	1.166269	20.89	47.0	1.21538	1.213238	25.70
38.2	1.16934	1.167281	21.00	47.2	1.21646	1.214317	25.80
38.4	1.17036	1.168293	21.11	47.4	1.21755	1.215395	25.91
38.6	1.17138	1.169307	21.21	47.6	1.21863	1.216476	26.01
38.8	1.17239	1.170322	21.32	47.8	1.21971	1.217559	26.12
39.0	1.17341	1.171340	21.43	48.0	1.22080	1.218643	26.23
39.2	1.17443	1.172359	21.54	48.2	1.22189	1.219729	26.33
39.4	1.17545	1.173379	21.64	48.4	1.22298	1.220815	26.44
39.6	1.17648	1.174400	21.75	48.6	1.22406	1.221904	26.54
39.8	1.17750	1.175423	21.86	48.8	1.22516	1.222995	26.65
40.0	1.17853	1.176447	21.97	49.0	1.22625	1.224086	26.75
40.2	1.17956	1.177473	22.07	49.2	1.22735	1.225180	26.86
40.4	1.18058	1.178501	22.18	49.4	1.22844	1.226274	26.96
40.6	1.18162	1.179527	22.29	49.6	1.22954	1.227371	27.07
40.8	1.18265	1.180560	22.39	49.8	1.23064	1.228469	27.18
41.0	1.18368	1.181592	22.50	50.0	1.23174	1.229567	27.28
41.2	1.18472	1.182625	22.61	50.2	1.23284	1.230668	27.39
41.4	1.18575	1.183660	22.72	50.4	1.23395	1.231770	27.49
41.6	1.18679	1.184696	22.82	50.6	1.23506	1.232874	27.60
41.8	1.18783	1.185734	22.93	50.8	1.23616	1.233979	27.70
42.0	1.18887	1.186773	23.04	51.0	1.23727	1.235085	27.81
42.2	1.18992	1.187814	23.14	51.2	1.23838	1.236194	27.91
42.4	1.19096	1.188856	23.25	51.4	1.23949	1.237303	28.02
42.6	1.19201	1.189901	23.36	51.6	1.24060	1.238414	28.12
42.8	1.19305	1.190946	23.46	51.8	1.24172	1.239527	28.23
43.0	1.19410	1.191993	23.57	52.0	1.24284	1.240641	28.33
43.2	1.19515	1.193041	23.68	52.2	1.24395	1.241757	28.44
43.4	1.19620	1.194090	23.78	52.4	1.24507	1.242873	28.54
43.6	1.19726	1.195141	23.89	52.6	1.24619	1.243992	28.65
43.8	1.19831	1.196193	24.00	52.8	1.24731	1.245113	28.75
44.0	1.19936	1.197247	24.10	53.0	1.24844	1.246234	28.86
44.2	1.20042	1.198303	24.21	53.2	1.24956	1.247358	28.96
44.4	1.20148	1.199360	24.32	53.4	1.25069	1.248482	29.06
44.6	1.20254	1.200420	24.42	53.6	1.25182	1.249609	29.17
44.8	1.20360	1.201480	24.53	53.8	1.25295	1.250737	29.27

*Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.—Continued.*

44.3

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
54.0	1.25408	1.251866	29.38	63.0	1.30657	1.304267	34.02
54.2	1.25521	1.252997	29.48	63.2	1.30778	1.305467	34.12
54.4	1.25635	1.254129	29.59	63.4	1.30898	1.306669	34.23
54.6	1.25748	1.255264	29.69	63.6	1.31019	1.307872	34.33
54.8	1.25862	1.256400	29.80	63.8	1.31139	1.309077	34.43
55.0	1.25976	1.257535	29.90	64.0	1.31260	1.310282	34.53
55.2	1.26090	1.258674	30.00	64.2	1.31381	1.311489	34.63
55.4	1.26204	1.259815	30.11	64.4	1.31502	1.312699	34.74
55.6	1.26319	1.260955	30.21	64.6	1.31623	1.313909	34.84
55.8	1.26433	1.262099	30.32	64.8	1.31745	1.315121	34.94
56.0	1.26548	1.263243	30.42	65.0	1.31866	1.316334	35.04
56.2	1.26663	1.264390	30.52	65.2	1.31988	1.317549	35.14
56.4	1.26778	1.265537	30.63	65.4	1.32110	1.318766	35.24
56.6	1.26893	1.266686	30.73	65.6	1.32232	1.319983	35.34
56.8	1.27008	1.267837	30.83	65.8	1.32354	1.321203	35.45
57.0	1.27123	1.268989	30.94	66.0	1.32476	1.322425	35.55
57.2	1.27239	1.270143	31.04	66.2	1.32599	1.323648	35.65
57.4	1.27355	1.271299	31.15	66.4	1.32722	1.324872	35.75
57.6	1.27471	1.272455	31.25	66.6	1.32844	1.326097	35.85
57.8	1.27587	1.273614	31.35	66.8	1.32967	1.327325	35.95
58.0	1.27703	1.274774	31.46	67.0	1.33090	1.328554	36.05
58.2	1.27819	1.275936	31.56	67.2	1.33214	1.329785	36.15
58.4	1.27936	1.277098	31.66	67.4	1.33337	1.331017	36.25
58.6	1.28052	1.278262	31.76	67.6	1.33460	1.332250	36.35
58.8	1.28169	1.279428	31.87	67.8	1.33584	1.333485	36.45
59.0	1.28286	1.280595	31.97	68.0	1.33708	1.334722	36.55
59.2	1.28404	1.281764	32.07	68.2	1.33832	1.335961	36.66
59.4	1.28520	1.282935	32.18	68.4	1.33957	1.337200	36.76
59.6	1.28638	1.284107	32.28	68.6	1.34081	1.338441	36.86
59.8	1.28755	1.285281	32.38	68.8	1.34205	1.339684	36.96
60.0	1.28873	1.286456	32.49	69.0	1.34330	1.340928	37.06
60.2	1.28991	1.287633	32.59	69.2	1.34455	1.342174	37.16
60.4	1.29109	1.288811	32.69	69.4	1.34580	1.343421	37.26
60.6	1.29227	1.289991	32.79	69.6	1.34705	1.344671	37.36
60.8	1.29346	1.291172	32.90	69.8	1.34830	1.345922	37.46
61.0	1.29464	1.292354	33.00	70.0	1.34956	1.347174	37.56
61.2	1.29583	1.293539	33.10	70.2	1.35081	1.348427	37.66
61.4	1.29701	1.294725	33.20	70.4	1.35207	1.349682	37.76
61.6	1.29820	1.295911	33.31	70.6	1.35333	1.350939	37.86
61.8	1.29940	1.297100	33.41	70.8	1.35459	1.352197	37.96
62.0	1.30059	1.298291	33.51	71.0	1.35585	1.353456	38.06
62.2	1.30178	1.299483	33.61	71.2	1.35711	1.354717	38.16
62.4	1.30298	1.300677	33.72	71.4	1.35838	1.355980	38.26
62.6	1.30418	1.301871	33.82	71.6	1.35964	1.357245	38.35
62.8	1.30537	1.303068	33.92	71.8	1.36091	1.358511	38.45

44.3 *Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.—Continued.*

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
72.0	1.36218	1.359778	38.55	81.0	1.42088	1.418374	42.95
72.2	1.36346	1.361047	38.65	81.2	1.42222	1.419711	43.05
72.4	1.36473	1.362317	38.75	81.4	1.42356	1.421049	43.14
72.6	1.36600	1.363590	38.85	81.6	1.42490	1.422390	43.24
72.8	1.36728	1.364864	38.95	81.8	1.42625	1.423730	43.33
73.0	1.36856	1.366139	39.05	82.0	1.42759	1.425072	43.43
73.2	1.36983	1.367415	39.15	82.2	1.42894	1.426416	43.53
73.4	1.37111	1.368693	39.25	82.4	1.43029	1.427761	43.62
73.6	1.37240	1.369973	39.35	82.6	1.43164	1.429109	43.72
73.8	1.37368	1.371254	39.44	82.8	1.43298	1.430457	43.81
74.0	1.37496	1.372536	39.54	83.0	1.43434	1.431807	43.91
74.2	1.37625	1.373820	39.64	83.2	1.43569	1.433158	44.00
74.4	1.37754	1.375105	39.74	83.4	1.43705	1.434511	44.10
74.6	1.37883	1.376392	39.84	83.6	1.43841	1.435866	44.19
74.8	1.38012	1.377680	39.94	83.8	1.43976	1.437222	44.29
75.0	1.38141	1.378971	40.03	84.0	1.44112	1.438579	44.38
75.2	1.38270	1.380262	40.13	84.2	1.44249	1.439938	44.48
75.4	1.38400	1.381555	40.23	84.4	1.44385	1.441299	44.57
75.6	1.38530	1.382851	40.33	84.6	1.44521	1.442661	44.67
75.8	1.38660	1.384148	40.43	84.8	1.44658	1.444024	44.76
76.0	1.38790	1.385446	40.53	85.0	1.44794	1.445388	44.86
76.2	1.38920	1.386745	40.62	85.2	1.44931	1.446754	44.95
76.4	1.39050	1.388045	40.72	85.4	1.45068	1.448121	45.05
76.6	1.39180	1.389347	40.82	85.6	1.45205	1.449491	45.14
76.8	1.39311	1.390651	40.92	85.8	1.45343	1.450860	45.24
77.0	1.39442	1.391956	41.01	86.0	1.45480	1.452232	45.33
77.2	1.39573	1.393263	41.11	86.2	1.45618	1.453605	45.42
77.4	1.39704	1.394571	41.21	86.4	1.45755	1.454980	45.52
77.6	1.39835	1.395881	41.31	86.6	1.45893	1.456357	45.61
77.8	1.39966	1.397192	41.40	86.8	1.46031	1.457735	45.71
78.0	1.40098	1.398505	41.50	87.0	1.46170	1.459114	45.80
78.2	1.40230	1.399819	41.60	87.2	1.46308	1.460495	45.89
78.4	1.40361	1.401134	41.70	87.4	1.46446	1.461877	45.99
78.6	1.40493	1.402452	41.79	87.6	1.46585	1.463260	46.08
78.8	1.40625	1.403771	41.89	87.8	1.46724	1.464645	46.17
79.0	1.40758	1.405091	41.99	88.0	1.46862	1.466032	46.27
79.2	1.40890	1.406412	42.08	88.2	1.47002	1.467420	46.36
79.4	1.41023	1.407735	42.18	88.4	1.47141	1.468810	46.45
79.6	1.41155	1.409061	42.28	88.6	1.47280	1.470200	46.55
79.8	1.41288	1.410387	42.37	88.8	1.47420	1.471592	46.64
80.0	1.41421	1.411715	42.47	89.0	1.47559	1.472986	46.73
80.2	1.41554	1.413044	42.57	89.2	1.47699	1.474381	46.83
80.4	1.41688	1.414374	42.66	89.4	1.47839	1.475779	46.92
80.6	1.41821	1.415706	42.76	89.6	1.47979	1.477176	47.01
80.8	1.41955	1.417039	42.85	89.8	1.48119	1.478575	47.11



*Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.—Concluded.*

44.3

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
90.0	1.48259	1.479976	47.20	95.0	1.51814	1.515455	49.49
90.2	1.48400	1.481378	47.29	95.2	1.51958	1.516893	49.58
90.4	1.48540	1.482782	47.38	95.4	1.52102	1.518332	49.67
90.6	1.48681	1.484187	47.48	95.6	1.52246	1.519771	49.76
90.8	1.48822	1.485593	47.57	95.8	1.52390	1.521212	49.85
91.0	1.48963	1.487002	47.66	96.0	1.52535	1.522656	49.94
91.2	1.49104	1.488411	47.75	96.2	1.52680	1.524100	50.03
91.4	1.49246	1.489823	47.84	96.4	1.52824	1.525546	50.12
91.6	1.49387	1.491234	47.94	96.6	1.52969	1.526993	50.21
91.8	1.49529	1.492647	48.03	96.8	1.53114	1.528441	50.30
92.0	1.49671	1.494063	48.12	97.0	1.53260	1.529891	50.39
92.2	1.49812	1.495479	48.21	97.2	1.53405	1.531342	50.48
92.4	1.49954	1.496897	48.30	97.4	1.53551	1.532794	50.57
92.6	1.50097	1.498316	48.40	97.6	1.53696	1.534248	50.66
92.8	1.50239	1.499736	48.49	97.8	1.53842	1.535704	50.75
93.0	1.50381	1.501158	48.58	98.0	1.53988	1.537161	50.84
93.2	1.50524	1.502582	48.67	98.2	1.54134	1.538618	50.93
93.4	1.50667	1.504006	48.76	98.4	1.54280	1.540076	51.02
93.6	1.50810	1.505432	48.85	98.6	1.54426	1.541536	51.10
93.8	1.50952	1.506859	48.94	98.8	1.54573	1.542998	51.19
94.0	1.51096	1.508289	49.03	99.0	1.54719	1.544462	51.28
94.2	1.51239	1.509720	49.12	99.2	1.54866	1.545926	51.37
94.4	1.51382	1.511151	49.22	99.4	1.55013	1.547392	51.46
94.6	1.51526	1.512585	49.31	99.6	1.55160	1.548861	51.55
94.8	1.51670	1.514019	49.40	99.8	1.55307	1.550329	51.64
				100.0	1.55454	1.551800	51.73

**44.4**      *Temperature corrections to readings of saccharimeters*  
(standard at 20°C).

(This table is calculated from the data on the thermal expansion of sugar solutions by Plato,<sup>1</sup> and it is assumed that the instrument is of Jena 16<sup>11</sup> glass. The table should be used with caution and only for approximate results when the temperature differs much from the standard temperature or from the temperature of the surrounding air.)

TEMPERATURE IN DEGREES CENTIGRADE	OBSERVED PERCENTAGE OF SUGAR													
	0	5	10	15	20	25	30	35	40	45	50	55	60	70
	Subtract—													
0	0.30	0.49	0.65	0.77	0.89	0.99	1.08	1.16	1.24	1.31	1.37	1.41	1.44	1.49
5	0.36	0.47	0.56	0.65	0.73	0.80	0.86	0.91	0.97	1.01	1.05	1.08	1.10	1.14
10	0.32	0.38	0.43	0.48	0.52	0.57	0.60	0.64	0.67	0.70	0.72	0.74	0.75	0.77
11	0.31	0.35	0.40	0.44	0.48	0.51	0.55	0.58	0.60	0.63	0.65	0.66	0.68	0.70
12	0.29	0.32	0.36	0.40	0.43	0.46	0.50	0.52	0.54	0.56	0.58	0.59	0.60	0.62
13	0.26	0.29	0.32	0.35	0.38	0.41	0.44	0.46	0.48	0.49	0.51	0.52	0.53	0.55
14	0.24	0.26	0.29	0.31	0.34	0.36	0.38	0.40	0.41	0.42	0.44	0.45	0.46	0.47
15	0.20	0.22	0.24	0.26	0.28	0.30	0.32	0.33	0.34	0.36	0.36	0.37	0.38	0.39
16	0.17	0.18	0.20	0.22	0.23	0.25	0.26	0.27	0.28	0.28	0.29	0.30	0.31	0.32
17	0.13	0.14	0.15	0.16	0.18	0.19	0.20	0.20	0.21	0.21	0.22	0.23	0.23	0.24
18	0.09	0.10	0.10	0.11	0.12	0.13	0.13	0.14	0.14	0.14	0.15	0.15	0.15	0.16
19	0.05	0.05	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08
17.5	0.11	0.12	0.12	0.14	0.15	0.16	0.16	0.17	0.17	0.18	0.18	0.19	0.19	0.20
15.56 (60°F)	0.18	0.20	0.22	0.24	0.26	0.28	0.29	0.30	0.30	0.32	0.33	0.33	0.34	0.34
	Add—													
21	0.04	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.09
22	0.10	0.10	0.11	0.12	0.12	0.13	0.14	0.14	0.15	0.15	0.16	0.16	0.16	0.16
23	0.16	0.16	0.17	0.17	0.19	0.20	0.21	0.21	0.22	0.23	0.24	0.24	0.24	0.24
24	0.21	0.22	0.23	0.24	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.32	0.32	0.32
25	0.27	0.28	0.30	0.31	0.32	0.34	0.35	0.36	0.38	0.38	0.39	0.39	0.40	0.39
26	0.33	0.34	0.36	0.37	0.40	0.40	0.42	0.44	0.46	0.47	0.47	0.48	0.48	0.48
27	0.40	0.41	0.42	0.44	0.46	0.48	0.50	0.52	0.54	0.54	0.55	0.56	0.56	0.56
28	0.46	0.47	0.49	0.51	0.54	0.56	0.58	0.60	0.61	0.62	0.63	0.64	0.64	0.64
29	0.54	0.55	0.56	0.59	0.61	0.63	0.66	0.68	0.70	0.70	0.71	0.72	0.72	0.72
30	0.61	0.62	0.63	0.66	0.68	0.71	0.73	0.76	0.78	0.78	0.79	0.80	0.80	0.81
35	0.99	1.01	1.02	1.06	1.10	1.13	1.16	1.18	1.20	1.21	1.22	1.22	1.23	1.22
40	1.42	1.45	1.47	1.51	1.54	1.57	1.60	1.62	1.64	1.65	1.65	1.65	1.66	1.65
45	1.91	1.94	1.96	2.00	2.03	2.05	2.07	2.09	2.10	2.10	2.10	2.10	2.10	2.08
50	2.46	2.48	2.50	2.53	2.56	2.57	2.58	2.59	2.59	2.58	2.58	2.57	2.56	2.52
55	3.05	3.07	3.09	3.12	3.12	3.12	3.12	3.11	3.10	3.08	3.07	3.05	3.03	2.97
60	3.69	3.72	3.73	3.73	3.72	3.70	3.67	3.65	3.62	3.60	3.57	3.54	3.50	3.43
27.5	0.43	0.44	0.46	0.48	0.50	0.52	0.54	0.56	0.58	0.58	0.59	0.60	0.60	0.60

<sup>1</sup> Wiss. Abh. Kaiserliche Normal-Eichungs-Kommission, Vol. 2, 1900, p. 140.

*Domke's table of apparent specific gravity of sucrose solutions at 20°C.<sup>1</sup>* 44.5

Calculated from the tables of the Kaiserliche Normal-Eichungs-Kommission and accepted by the International Commission for Uniform Methods of Sugar Analysis.

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
0	1.0000	1.0004	1.0008	1.0012	1.0016	1.0019	1.0023	1.0027	1.0031	1.0035
1	1.0039	1.0043	1.0047	1.0051	1.0055	1.0058	1.0062	1.0066	1.0070	1.0074
2	1.0078	1.0082	1.0086	1.0090	1.0094	1.0098	1.0102	1.0106	1.0109	1.0113
3	1.0117	1.0121	1.0125	1.0129	1.0133	1.0137	1.0141	1.0145	1.0149	1.0153
4	1.0157	1.0161	1.0165	1.0169	1.0173	1.0177	1.0181	1.0185	1.0189	1.0193
5	1.0197	1.0201	1.0205	1.0209	1.0213	1.0217	1.0221	1.0225	1.0229	1.0233
6	1.0237	1.0241	1.0245	1.0249	1.0253	1.0257	1.0261	1.0265	1.0269	1.0273
7	1.0277	1.0281	1.0285	1.0289	1.0294	1.0298	1.0302	1.0306	1.0310	1.0314
8	1.0318	1.0322	1.0326	1.0330	1.0334	1.0338	1.0343	1.0347	1.0351	1.0355
9	1.0359	1.0363	1.0367	1.0371	1.0375	1.0380	1.0384	1.0388	1.0392	1.0396
10	1.0400	1.0404	1.0409	1.0413	1.0417	1.0421	1.0425	1.0429	1.0433	1.0438
11	1.0442	1.0446	1.0450	1.0454	1.0459	1.0463	1.0467	1.0471	1.0475	1.0480
12	1.0484	1.0488	1.0492	1.0496	1.0501	1.0505	1.0509	1.0513	1.0517	1.0522
13	1.0526	1.0530	1.0534	1.0539	1.0543	1.0547	1.0551	1.0556	1.0560	1.0564
14	1.0568	1.0573	1.0577	1.0581	1.0585	1.0589	1.0594	1.0598	1.0603	1.0607
15	1.0611	1.0615	1.0620	1.0624	1.0628	1.0633	1.0637	1.0641	1.0646	1.0650
16	1.0654	1.0659	1.0663	1.0667	1.0672	1.0676	1.0680	1.0685	1.0689	1.0693
17	1.0698	1.0702	1.0706	1.0711	1.0715	1.0719	1.0724	1.0728	1.0733	1.0737
18	1.0741	1.0746	1.0750	1.0755	1.0759	1.0763	1.0768	1.0772	1.0777	1.0781
19	1.0785	1.0790	1.0794	1.0799	1.0803	1.0807	1.0812	1.0816	1.0821	1.0825
20	1.0830	1.0834	1.0839	1.0843	1.0848	1.0852	1.0856	1.0861	1.0865	1.0870
21	1.0874	1.0879	1.0883	1.0888	1.0892	1.0897	1.0901	1.0905	1.0910	1.0915
22	1.0919	1.0924	1.0928	1.0933	1.0937	1.0942	1.0946	1.0951	1.0956	1.0960
23	1.0965	1.0969	1.0974	1.0978	1.0983	1.0987	1.0992	1.0997	1.1001	1.1006
24	1.1010	1.1015	1.1020	1.1024	1.1029	1.1033	1.1038	1.1043	1.1047	1.1052
25	1.1056	1.1061	1.1066	1.1070	1.1075	1.1079	1.1084	1.1089	1.1093	1.1098
26	1.1103	1.1107	1.1112	1.1117	1.1121	1.1126	1.1131	1.1135	1.1140	1.1145
27	1.1149	1.1154	1.1159	1.1163	1.1168	1.1173	1.1178	1.1182	1.1187	1.1192
28	1.1196	1.1201	1.1206	1.1210	1.1215	1.1220	1.1225	1.1229	1.1234	1.1239
29	1.1244	1.1248	1.1253	1.1258	1.1263	1.1267	1.1272	1.1277	1.1282	1.1287
30	1.1291	1.1296	1.1301	1.1306	1.1311	1.1315	1.1320	1.1325	1.1330	1.1334
31	1.1339	1.1344	1.1349	1.1354	1.1359	1.1363	1.1368	1.1373	1.1378	1.1383
32	1.1388	1.1393	1.1397	1.1402	1.1407	1.1412	1.1417	1.1422	1.1427	1.1432
33	1.1436	1.1441	1.1446	1.1451	1.1456	1.1461	1.1466	1.1471	1.1476	1.1481
34	1.1486	1.1490	1.1495	1.1500	1.1505	1.1510	1.1515	1.1520	1.1525	1.1530
35	1.1535	1.1540	1.1545	1.1550	1.1555	1.1560	1.1565	1.1570	1.1575	1.1580
36	1.1585	1.1590	1.1595	1.1600	1.1605	1.1610	1.1615	1.1620	1.1625	1.1630
37	1.1635	1.1640	1.1645	1.1650	1.1655	1.1660	1.1665	1.1670	1.1675	1.1680
38	1.1685	1.1690	1.1696	1.1701	1.1706	1.1711	1.1716	1.1721	1.1726	1.1731
39	1.1736	1.1741	1.1746	1.1752	1.1757	1.1762	1.1767	1.1772	1.1777	1.1782
40	1.1787	1.1793	1.1798	1.1803	1.1808	1.1813	1.1818	1.1824	1.1829	1.1834
41	1.1839	1.1844	1.1849	1.1855	1.1860	1.1865	1.1870	1.1875	1.1881	1.1886
42	1.1891	1.1896	1.1901	1.1907	1.1912	1.1917	1.1922	1.1928	1.1933	1.1938
43	1.1943	1.1949	1.1954	1.1959	1.1964	1.1970	1.1975	1.1980	1.1985	1.1991
44	1.1996	1.2001	1.2007	1.2012	1.2017	1.2023	1.2028	1.2033	1.2039	1.2044

<sup>1</sup> Z. Ver. deut. Zucker-Ind., 62, 306 (1912).



*Jackson-Mathews table of densities of levulose solutions and mean density and expansion coefficients between 20° and 25°C.<sup>1</sup>*

44.6

(All weights corrected to vacuum.)

LEVU- LOSE, PER CENT	D <sub>t</sub> <sup>20</sup>	D <sub>t</sub> <sup>25</sup>	-ΔD/Δt	Δv/Δt	LEVU- LOSE, PER CENT	D <sub>t</sub> <sup>20</sup>	D <sub>t</sub> <sup>25</sup>	-ΔD/Δt	Δv/Δt
			×10 <sup>-6</sup>	×10 <sup>-6</sup>				×10 <sup>-6</sup>	×10 <sup>-5</sup>
0	0.99823	0.99708	231	231	36	1.1568	1.1544	48	42
1	1.00214	1.00095	238	237	37	1.1618	1.1593	49	42
2	1.00607	1.00484	245	243	38	1.1668	1.1643	50	43
3	1.01003	1.00877	252	249	39	1.1718	1.1693	50	43
4	1.01402	1.01272	259	255	40	1.1769	1.17435	51	43
5	1.01803	1.01670	266	261	41	1.1820	1.1794	52	44
6	1.02207	1.02071	273	267	42	1.1872	1.1845	53	44
7	1.02614	1.02475	280	273	43	1.1923	1.1897	53	45
8	1.03024	1.02881	287	278	44	1.1975	1.19485	54	45
9	1.03437	1.03290	294	284	45	1.2028	1.20005	55	45
10	1.03853	1.03702	301	290	46	1.20805	1.2053	55	46
11	1.04271	1.04118	308	295	47	1.2134	1.2106	56	46
12	1.04692	1.04535	315	300	48	1.2187	1.2159	57	46
13	1.05116	1.04955	323	307	49	1.2241	1.2212	57	47
14	1.05543	1.05378	330	313	50	1.2295	1.2266	58	47
15	1.05972	1.05804	337	318	51	1.2349	1.2320	59	47
16	1.06405	1.06233	345	324	52	1.2404	1.2374	59	48
17	1.06840	1.06664	352	329	53	1.2459	1.2429	60	48
18	1.07278	1.07098	360	336	54	1.2514	1.2484	60	48
19	1.07719	1.07535	367	341	55	1.2570	1.2539	61	49
20	1.08162	1.07975	375	347	56	1.2626	1.2595	62	49
21	1.08606	1.0842	38	35	57	1.2682	1.2651	62	49
22	1.09055	1.0886	38	35	58	1.2739	1.2707	63	50
23	1.09507	1.0931	39	36	59	1.2796	1.2764	64	50
24	1.09962	1.0976	40	36	60	1.2853	1.2821	64	50
25	1.10420	1.1022	41	37	61	1.2911	1.2878	65	50
26	1.1088	1.10675	41	37	62	1.2969	1.2936	66	51
27	1.11345	1.11135	42	38	63	1.3027	1.2994	66	51
28	1.1181	1.1160	43	38	64	1.3086	1.3052	67	51
29	1.1229	1.1207	43	39	65	1.3145	1.3111	67	51
30	1.1276	1.1254	44	39	66	1.3204	1.3170	68	51
31	1.1324	1.13015	45	40	67	1.3263	1.3229	69	52
32	1.1372	1.1349	46	40	68	1.3323	1.3289	69	52
33	1.14205	1.1397	46	40	69	1.3384	1.3349	70	52
34	1.1469	1.1446	47	41	70	1.3444	1.3409	70	52
35	1.15185	1.1495	48	41	71	1.3505	1.3470	71	53

<sup>1</sup> J. Research, Nat. Bur. Standards, 8, 437 (1932), RP 426; Circ. 440 Nat. Bur. Standards.

44.7

*Refractive indices of sucrose solutions at 20°C.<sup>1</sup>*  
(International Scale, 1936)<sup>2</sup>

REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT
1.33299	0.0	1.34629	9.0	1.36053	18.0	1.3758	27.0	1.3920	36.0
.33328	0.2	.34660	9.2	.36086	18.2	.3761	27.2	.3924	36.2
.33357	0.4	.34691	9.4	.36119	18.4	.3765	27.4	.3928	36.4
.33385	0.6	.34721	9.6	.36152	18.6	.3768	27.6	.3931	36.6
.33414	0.8	.34752	9.8	.36185	18.8	.3772	27.8	.3935	36.8
.33443	1.0	.34783	10.0	.36218	19.0	.3775	28.0	.3939	37.0
.33472	1.2	.34814	10.2	.36251	19.2	.3779	28.2	.3943	37.2
.33501	1.4	.34845	10.4	.36284	19.4	.3782	28.4	.3947	37.4
.33530	1.6	.34875	10.6	.36318	19.6	.3786	28.6	.3950	37.6
.33559	1.8	.34906	10.8	.36351	19.8	.3789	28.8	.3954	37.8
.33588	2.0	.34937	11.0	.36384	20.0	.3793	29.0	.3958	38.0
.33617	2.2	.34968	11.2	.36417	20.2	.3797	29.2	.3962	38.2
.33646	2.4	.34999	11.4	.36451	20.4	.3800	29.4	.3966	38.4
.33675	2.6	.35031	11.6	.36484	20.6	.3804	29.6	.3970	38.6
.33704	2.8	.35062	11.8	.36518	20.8	.3807	29.8	.3974	38.8
.33733	3.0	.35093	12.0	.36551	21.0	.3811	30.0	.3978	39.0
.33762	3.2	.35124	12.2	.36585	21.2	.3815	30.2	.3982	39.2
.33792	3.4	.35156	12.4	.36618	21.4	.3818	30.4	.3986	39.4
.33821	3.6	.35187	12.6	.36652	21.6	.3822	30.6	.3989	39.6
.33851	3.8	.35219	12.8	.36685	21.8	.3825	30.8	.3993	39.8
.33880	4.0	.35250	13.0	.36719	22.0	.3829	31.0	.3997	40.0
.33909	4.2	.35282	13.2	.36753	22.2	.3833	31.2	.4001	40.2
.33939	4.4	.35313	13.4	.36787	22.4	.3836	31.4	.4005	40.4
.33968	4.6	.35345	13.6	.36820	22.6	.3840	31.6	.4008	40.6
.33998	4.8	.35376	13.8	.36854	22.8	.3843	31.8	.4012	40.8
.34027	5.0	.35408	14.0	.36888	23.0	.3847	32.0	.4016	41.0
.34057	5.2	.35440	14.2	.36922	23.2	.3851	32.2	.4020	41.2
.34087	5.4	.35472	14.4	.36956	23.4	.3854	32.4	.4024	41.4
.34116	5.6	.35503	14.6	.36991	23.6	.3858	32.6	.4028	41.6
.34146	5.8	.35535	14.8	.37025	23.8	.3861	32.8	.4032	41.8
.34176	6.0	.35567	15.0	.37059	24.0	.3865	33.0	.4036	42.0
.34206	6.2	.35599	15.2	.3709	24.2	.3869	33.2	.4040	42.2
.34236	6.4	.35631	15.4	.3713	24.4	.3872	33.4	.4044	42.4
.34266	6.6	.35664	15.6	.3716	24.6	.3876	33.6	.4048	42.6
.34296	6.8	.35696	15.8	.3720	24.8	.3879	33.8	.4052	42.8
.34326	7.0	.35728	16.0	.3723	25.0	.3883	34.0	.4056	43.0
.34356	7.2	.35760	16.2	.3726	25.2	.3887	34.2	.4060	43.2
.34386	7.4	.35793	16.4	.3730	25.4	.3891	34.4	.4064	43.4
.34417	7.6	.35825	16.6	.3733	25.6	.3894	34.6	.4068	43.6
.34447	7.8	.35858	16.8	.3737	25.8	.3898	34.8	.4072	43.8
.34477	8.0	.35890	17.0	.3740	26.0	.3902	35.0	.4076	44.0
.34507	8.2	.35923	17.2	.3744	26.2	.3906	35.2	.4080	44.2
.34538	8.4	.35955	17.4	.3747	26.4	.3909	35.4	.4084	44.4
.34568	8.6	.35988	17.6	.3751	26.6	.3913	35.6	.4088	44.6
.34599	8.8	.36020	17.8	.3754	26.8	.3916	35.8	.4092	44.8

<sup>1</sup> This table is in accordance with the International Scale of Refractive Indices of Sucrose Solutions at 20°C, 1936, adopted as official (final action) at the 1938 meeting of the Association. The values of the indices for the range 0–24% sucrose are given to five decimal places instead of to four. This arrangement is desirable when the table is used with refractometers capable of readings to the fifth place. The values for whole per cents of sucrose are those of the International Scale; the remaining fractional values are obtained by interpolation. The values of indices above 24% sucrose are identical with those in Table 6, *Methods of Analysis*, A. O. A. C., 1935, p. 622.

<sup>2</sup> *Intern. Sugar J.*, 39, 22s (1937).



**44.8** *Corrections for determining percentage of sucrose in sugar solutions by means of either Abbé or immersion refractometer when readings are made at temperatures other than 20°C.<sup>1</sup>*

(International Temperature Correction Table, 1936)<sup>1</sup>

TEMP. °C	PER CENT SUCROSE										
	0	5	10	15	20	25	30	40	50	60	70
	Subtract from the per cent sucrose										
10	0.50	0.54	0.58	0.61	0.64	0.66	0.68	0.72	0.74	0.76	0.79
11	.46	.49	.53	.55	.58	.60	.62	.65	.67	.69	.71
12	.42	.45	.48	.50	.52	.54	.56	.58	.60	.61	.63
13	.37	.40	.42	.44	.46	.48	.49	.51	.53	.54	.55
14	.33	.35	.37	.39	.40	.41	.42	.44	.45	.46	.48
15	.27	.29	.31	.33	.34	.34	.35	.37	.38	.39	.40
16	.22	.24	.25	.26	.27	.28	.28	.30	.30	.31	.32
17	.17	.18	.19	.20	.21	.21	.21	.22	.23	.23	.24
18	.12	.13	.13	.14	.14	.14	.14	.15	.15	.16	.16
19	.06	.06	.06	.07	.07	.07	.07	.08	.08	.08	.08
	Add to the per cent sucrose										
21	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08
22	.13	.13	.14	.14	.15	.15	.15	.15	.16	.16	.16
23	.19	.20	.21	.22	.22	.23	.23	.23	.24	.24	.24
24	.26	.27	.28	.29	.30	.30	.31	.31	.31	.32	.32
25	.33	.35	.36	.37	.38	.38	.39	.40	.40	.40	.40
26	.40	.42	.43	.44	.45	.46	.47	.48	.48	.48	.48
27	.48	.50	.52	.53	.54	.55	.55	.56	.56	.56	.56
28	.56	.57	.60	.61	.62	.63	.63	.64	.64	.64	.64
29	.64	.66	.68	.69	.71	.72	.72	.73	.73	.73	.73
30	.72	.74	.77	.78	.79	.80	.80	.81	.81	.81	.81

<sup>1</sup> Intern. Sugar J., 39, 248 (1937).



44.9 Table for determining percentage of sucrose in sugar solutions from readings of Zeiss immersion refractometer at 20°C.<sup>1</sup>

SCALE READING <sup>2</sup> 20°C.	$n_D^{20}$	SUCROSE PER CENT	SCALE READING <sup>2</sup> 20°C.	$n_D^{20}$	SUCROSE PER CENT	SCALE READING <sup>2</sup> 20°C.	$n_D^{20}$	SUCROSE PER CENT
14.47	1.33299	0	45	1.34463	7.91	76	1.35606	15.24
15	3320	0.15	46	4500	8.15	77	5642	15.47
16	3358	0.41	47	4537	8.39	78	5678	15.69
17	3397	0.68	48	4575	8.64	79	5714	15.91
18	3435	0.94	49	4612	8.89	80	5750	16.14
19	3474	1.21	50	4650	9.13	81	5786	16.36
20	3513	1.48	51	4687	9.38	82	5822	16.58
21	3551	1.74	52	4724	9.62	83	5858	16.81
22	3590	2.01	53	4761	9.86	84	5894	17.03
23	3628	2.27	54	4798	10.10	85	5930	17.25
24	3667	2.54	55	4836	10.34	86	5966	17.47
25	3705	2.80	56	4873	10.58	87	6002	17.69
26	3743	3.07	57	4910	10.82	88	6038	17.91
27	3781	3.33	58	4947	11.06	89	6074	18.12
28	3820	3.59	59	4984	11.30	90	6109	18.34
29	3858	3.85	60	5021	11.54	91	6145	18.56
30	3896	4.11	61	5058	11.78	92	6181	18.78
31	3934	4.36	62	5095	12.01	93	6217	19.00
32	3972	4.62	63	5132	12.25	94	6252	19.21
33	4010	4.88	64	5169	12.48	95	6287	19.42
34	4048	5.14	65	5205	12.72	96	6323	19.63
35	4086	5.40	66	5242	12.95	97	6359	19.85
36	4124	5.65	67	5279	13.18	98	6394	20.06
37	4162	5.91	68	5316	13.41	99	6429	20.27
38	4199	6.16	69	5352	13.64	100	6464	20.48
39	4237	6.41	70	5388	13.87	101	6500	20.69
40	4275	6.66	71	5425	14.10	102	6535	20.90
41	4313	6.91	72	5461	14.33	103	6570	21.11
42	4350	7.16	73	5497	14.56	104	6605	21.32
43	4388	7.41	74	5533	14.79	105	6640	21.53
44	4426	7.66	75	5569	15.01			

<sup>1</sup> The values in this table were calculated by J. A. Mathews from the five-place indices of Schönrock as given by Landt, *Z. Ver. deut. Zucker-Ind.*, 83, 692 (1933).

<sup>2</sup> The scale readings refer only to the scale of arbitrary units proposed by Pulfrich, *Z. angew. Chem.*, p. 1168 (1899). According to this scale 14.5 = 1.33300, 50.0 = 1.34650, and 100.0 = 1.36464. If the immersion refractometer used is calibrated according to another arbitrary scale, the readings must be converted into refractive indices before this table is used to determine the percentage of sugar.

## 44.10

*Refractive indices and Zeiss immersion refractometer readings of levulose solutions at 20° and 25°C.<sup>1</sup>*

LEVULOSE, PER CENT	$n_D^{20}$	ZEISS IMMER- SION READ- ING, 20° C.	$n_D^{25}$	ZEISS IMMER- SION READ- ING, 25° C.	LEVULOSE, PER CENT	$n_D^{20}$	$n_D^{25}$	LEVULOSE, PER CENT	$n_D^{20}$	$n_D^{25}$
0	1.33300	14.50	1.33252	13.25	32	1.38385	1.38297	64	1.4479	1.4467
1	1.33442	18.18	1.33393	16.90	33	1.38564	1.38476	65	1.4501	1.4489
2	1.33585	21.87	1.33535	20.58	34	1.38745	1.38655	66	1.4524	1.4512
3	1.33729	25.63	1.33678	24.29	35	1.38927	1.38836	67	1.4547	1.4535
4	1.33874	29.42	1.33822	28.05	36	1.39111	1.39018	68	1.4569	1.4557
5	1.34020	33.26	1.33967	31.87	37	1.39295	1.39201	69	1.4592	1.4580
6	1.34167	37.14	1.34113	35.71	38	1.39481	1.39386	70	1.4615	1.4602
7	1.34315	41.05	1.34260	39.61	39	1.39669	1.39573	71	1.4638	1.4625
8	1.34464	45.03	1.34408	43.53	40	1.39858	1.39760	72	1.4661	1.4648
9	1.34614	49.05	1.34557	47.53	41	1.40048	1.39949	73	1.4684	1.4672
10	1.34765	53.11	1.34707	51.54	42	1.40239	1.40140	74	1.4708	1.4695
11	1.34917	57.19	1.34857	55.57	43	1.40432	1.40331	75	1.4731	1.4719
12	1.35070	61.32	1.35009	59.68	44	1.40625	1.40524	76	1.4755	1.4742
13	1.35224	65.51	1.35162	63.81	45	1.40821	1.40718	77	1.4779	1.4766
14	1.35379	69.75	1.35316	68.00	46	1.41018	1.40914	78	1.4803	1.4790
15	1.35534	74.03	1.35470	72.25	47	1.41216	1.41111	79	1.4827	1.4814
16	1.35691	78.36	1.35626	76.56	48	1.41415	1.41309	80	1.4851	1.4838
17	1.35849	82.75	1.35783	80.92	49	1.41616	1.41509	81	1.4875	1.4862
18	1.36008	87.17	1.35942	85.28	50	1.41818	1.41710	82	1.4900	1.4887
19	1.36169	91.64	1.36102	89.71	51	1.42021	1.41912	83	1.4924	1.4912
20	1.36332	96.14	1.36262	94.17	52	1.42226	1.42117	84	1.4949	1.4936
21	1.36496	100.72	1.36425	98.71	53	1.42432	1.42322	85	1.4974	1.4961
22	1.36659	105.37	1.36588	103.31	54	1.42640	1.42528	86	1.4999	1.4986
23	1.36827	—	1.36753	—	55	1.42848	1.42736	87	1.5024	1.5011
24	1.36996	—	1.36921	—	56	1.43058	1.42945	88	1.5049	1.5036
25	1.37166	—	1.37088	—	57	1.43270	1.43156	89	1.5074	1.5062
26	1.37336	—	1.37258	—	58	1.43482	1.43368	90	1.5100	1.5087
27	1.37506	—	1.37426	—	59	1.43696	1.43581	91	1.5126	1.5113
28	1.37680	—	1.37598	—	60	1.43913	1.43797	92	1.5151	1.5139
29	1.37854	—	1.37771	—	61	1.44130	1.44014	93	1.5177	1.5165
30	1.38030	—	1.37945	—	62	1.44348	1.44232	94	1.5203	1.5191
31	1.38207	—	1.38121	—	63	1.44569	1.44451	95	1.5230	1.5217

<sup>1</sup> Jackson and Mathews, J. Research, Nat. Bur. Standards, 8, 437 (1932). RP 426; Circ. 440 Nat. Bur. Standards.

*Munson and Walker's table for calculating dextrose, invert sugar alone, invert sugar in the presence of sucrose (0.4 gram and 2 grams total sugar), lactose, lactose and sucrose (2 mixtures), and maltose (crystallized).<sup>1</sup>* **44.11**

(Applicable when  $\text{Cu}_2\text{O}$  is weighed directly)  
(Expressed in milligrams)

CUPROUS OXIDE ( $\text{Cu}_2\text{O}$ )	DEXTROROSE ( $d$ -GLUCOSE)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE $\text{C}_6\text{H}_{12}\text{O}_{11} + \text{H}_2\text{O}$	LACTOSE AND SUCROSE		MALTOSE $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	CUPROUS OXIDE ( $\text{Cu}_2\text{O}$ )
			0.4 gram total sugar	2 grams total sugar		1 lactose, 4 su- crose	1 lactose, 12 su- crose		
10	4.0	4.5	1.6	.....	6.3	6.1	.....	6.2	10
12	4.9	5.4	2.5	.....	7.5	7.3	.....	7.9	12
14	5.7	6.3	3.4	.....	8.8	8.5	.....	9.5	14
16	6.6	7.2	4.3	.....	10.0	9.7	.....	11.2	16
18	7.5	8.1	5.2	.....	11.3	10.9	.....	12.9	18
20	8.3	8.9	6.1	.....	12.5	12.1	.....	14.6	20
22	9.2	9.8	7.0	.....	13.8	13.3	.....	16.2	22
24	10.0	10.7	7.9	.....	15.0	14.5	.....	17.9	24
26	10.9	11.6	8.8	.....	16.3	15.8	.....	19.6	26
28	11.8	12.5	9.7	.....	17.6	17.0	.....	21.2	28
30	12.6	13.4	10.7	4.3	18.8	18.2	.....	22.9	30
32	13.5	14.3	11.6	5.2	20.1	19.4	.....	24.6	32
34	14.3	15.2	12.5	6.1	21.4	20.7	.....	26.2	34
36	15.2	16.1	13.4	7.0	22.8	22.0	.....	27.9	36
38	16.1	16.9	14.3	7.9	24.2	23.3	.....	29.6	38
40	16.9	17.8	15.2	8.8	25.5	24.7	.....	31.3	40
42	17.8	18.7	16.1	9.7	26.9	26.0	.....	32.9	42
44	18.7	19.6	17.0	10.7	28.3	27.3	.....	34.6	44
46	19.6	20.5	17.9	11.6	29.6	28.6	.....	36.3	46
48	20.4	21.4	18.8	12.5	31.0	30.0	.....	37.9	48
50	21.3	22.3	19.7	13.4	32.3	31.3	.....	39.6	50
52	22.2	23.2	20.7	14.3	33.7	32.6	.....	41.3	52
54	23.0	24.1	21.6	15.2	35.1	34.0	.....	42.9	54
56	23.9	25.0	22.5	16.2	36.4	35.3	.....	44.6	56
58	24.8	25.9	23.4	17.1	37.8	36.6	.....	46.3	58
60	25.6	26.8	24.3	18.0	39.2	37.9	.....	48.0	60
62	26.5	27.7	25.2	18.9	40.5	39.3	.....	49.6	62
64	27.4	28.6	26.2	19.8	41.9	40.6	.....	51.3	64
66	28.3	29.5	27.1	20.8	43.3	41.9	.....	53.0	66
68	29.2	30.4	28.0	21.7	44.7	43.3	40.7	54.6	68
70	30.0	31.3	28.9	22.6	46.0	44.6	41.9	56.3	70
72	30.9	32.3	29.8	23.5	47.4	45.9	43.1	58.0	72
74	31.8	33.2	30.8	24.5	48.8	47.3	44.2	59.6	74
76	32.7	34.1	31.7	25.4	50.1	48.6	45.4	61.3	76
78	33.6	35.0	32.6	26.3	51.5	49.9	46.6	63.0	78
80	34.4	35.9	33.5	27.3	52.9	51.3	47.8	64.6	80
82	35.3	36.8	34.5	28.2	54.2	52.6	49.0	66.3	82
84	36.2	37.7	35.4	29.1	55.6	53.9	50.1	68.0	84
86	37.1	38.6	36.3	30.0	57.0	55.3	51.3	69.7	86
88	38.0	39.5	37.2	31.0	58.4	56.6	52.5	71.3	88

<sup>1</sup> U. S. Bur. Standards Circ. 44, p. 139. The columns headed "Lactose" and "Lactose and Sucrose" were taken from "Methods of Sugar Analysis and Allied Determinations" by Arthur Given.

## 44.11

*Munson and Walker's table.*—Continued.  
(Expressed in milligrams.)

CUPROUS OXIDE (Cu <sub>2</sub> O)	DEXTROSE (d-GLUCOSE)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	LACTOSE AND SUCROSE		MALTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	CUPROUS OXIDE (Cu <sub>2</sub> O)
			0.4 gram total sugar	2 grams total sugar		1 lactose, 4 sucrose	1 lactose, 12 sucrose		
90	38.9	40.4	38.2	31.9	59.7	57.9	53.7	73.0	90
92	39.8	41.4	39.1	32.8	61.1	59.3	54.9	74.7	92
94	40.6	42.3	40.0	33.8	62.5	60.6	56.0	76.3	94
96	41.5	43.2	41.0	34.7	63.8	61.9	57.2	78.0	96
98	42.4	44.1	41.9	35.6	65.2	63.3	58.4	79.7	98
100	43.3	45.0	42.8	36.6	66.6	64.6	59.6	81.3	100
102	44.2	46.0	43.8	37.5	68.0	66.0	60.8	83.0	102
104	45.1	46.9	44.7	38.5	69.3	67.3	62.0	84.7	104
106	46.0	47.8	45.6	39.4	70.7	68.6	63.2	86.3	106
108	46.9	48.7	46.6	40.3	72.1	70.0	64.4	88.0	108
110	47.8	49.6	47.5	41.3	73.5	71.3	65.6	89.7	110
112	48.7	50.6	48.4	42.2	74.8	72.6	66.7	91.3	112
114	49.6	51.5	49.4	43.2	76.2	74.0	67.9	93.0	114
116	50.5	52.4	50.3	44.1	77.6	75.3	69.1	94.7	116
118	51.4	53.3	51.2	45.0	79.0	76.7	70.3	96.4	118
120	52.3	54.3	52.2	46.0	80.3	78.0	71.5	98.0	120
122	53.2	55.2	53.1	46.9	81.7	79.3	72.7	99.7	122
124	54.1	56.1	54.1	47.9	83.1	80.7	73.9	101.4	124
126	55.0	57.0	55.0	48.8	84.5	82.0	75.1	103.0	126
128	55.9	58.0	55.9	49.8	85.8	83.4	76.3	104.7	128
130	56.8	58.9	56.9	50.7	87.2	84.7	77.5	106.4	130
132	57.7	59.8	57.8	51.7	88.6	86.0	78.7	108.0	132
134	58.6	60.8	58.8	52.6	90.0	87.4	79.7	109.7	134
136	59.5	61.7	59.7	53.6	91.3	88.7	81.1	111.4	136
138	60.4	62.6	60.7	54.5	92.7	90.1	82.3	113.0	138
140	61.3	63.6	61.6	55.5	94.1	91.4	83.5	114.7	140
142	62.2	64.5	62.6	56.4	95.5	92.8	84.7	116.4	142
144	63.1	65.4	63.5	57.4	96.8	94.1	85.9	118.0	144
146	64.0	66.4	64.5	58.3	98.2	95.4	87.1	119.7	146
148	65.0	67.3	65.4	59.3	99.6	96.8	88.3	121.4	148
150	65.9	68.3	66.4	60.2	101.0	98.1	89.5	123.0	150
152	66.8	69.2	67.3	61.2	102.3	99.5	90.8	124.7	152
154	67.7	70.1	68.3	62.1	103.7	100.8	92.0	126.4	154
156	68.6	71.1	69.2	63.1	105.1	102.2	93.2	128.0	156
158	69.5	72.0	70.2	64.1	106.5	103.5	94.4	129.7	158
160	70.4	73.0	71.2	65.0	107.9	104.8	95.6	131.4	160
162	71.4	73.9	72.1	66.0	109.2	106.2	96.8	133.0	162
164	72.3	74.9	73.1	66.9	110.6	107.5	98.0	134.7	164
166	73.2	75.8	74.0	67.9	112.0	108.9	99.2	136.4	166
168	74.1	76.8	75.0	68.8	113.4	110.2	100.4	138.0	168

*Munson and Walker's table.*—Continued.  
(Expressed in milligrams.)

CUTEROUS OXIDE (CuO)	DEXTRINE (d-GLUCOSE)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE $C_{12}H_{22}O_{11}+H_2O$	LACTOSE AND SUCROSE		MALTOSE $C_{12}H_{22}O_{11}+H_2O$	CUTEROUS OXIDE (CuO)
			0.4 gram total sugar	2 grams total sugar		1 lactose, 4 sucrose	1 lactose, 12 sucrose		
170	75.1	77.7	76.0	69.8	114.8	111.6	101.6	139.7	170
172	76.0	78.7	76.9	70.8	116.1	112.9	102.8	141.4	172
174	76.9	79.6	77.9	71.7	117.5	114.3	104.1	143.0	174
176	77.8	80.6	78.8	72.7	118.9	115.6	105.3	144.7	176
178	78.8	81.5	79.8	73.7	120.3	117.0	106.5	146.4	178
180	79.7	82.5	80.8	74.6	121.6	118.3	107.7	148.0	180
182	80.6	83.4	81.7	75.6	123.1	119.7	108.9	149.7	182
184	81.5	84.4	82.7	76.6	124.3	121.0	110.1	151.4	184
186	82.5	85.3	83.7	77.6	125.8	122.4	111.3	153.0	186
188	83.4	86.3	84.6	78.5	127.2	123.7	112.5	154.7	188
190	84.3	87.2	85.6	79.5	128.5	125.1	113.8	156.4	190
192	85.3	88.2	86.6	80.5	129.9	126.4	115.0	158.0	192
194	86.2	89.2	87.6	81.4	131.3	127.8	116.2	159.7	194
196	87.1	90.1	88.5	82.4	132.7	129.2	117.4	161.4	196
198	88.1	91.1	89.5	83.4	134.1	130.5	118.6	163.0	198
200	89.0	92.0	90.5	84.4	135.4	131.9	119.8	164.7	200
202	89.9	93.0	91.4	85.3	136.8	133.2	121.0	166.4	202
204	90.9	94.0	92.4	86.3	138.2	134.6	122.3	168.0	204
206	91.8	94.9	93.4	87.3	139.6	135.9	123.5	169.7	206
208	92.8	95.9	94.4	88.3	141.0	137.3	124.7	171.4	208
210	93.7	96.9	95.4	89.2	142.3	138.6	126.0	173.0	210
212	94.6	97.8	96.3	90.2	143.7	140.0	127.2	174.7	212
214	95.6	98.8	97.3	91.2	145.1	141.4	128.4	176.4	214
216	96.5	99.8	98.3	92.2	146.5	142.7	129.6	178.0	216
218	97.5	100.8	99.3	93.2	147.9	144.1	130.9	179.7	218
220	98.4	101.7	100.3	94.2	149.3	145.4	132.1	181.4	220
222	99.4	102.7	101.2	95.1	150.7	146.8	133.3	183.0	222
224	100.3	103.7	102.2	96.1	152.0	148.1	134.5	184.7	224
226	101.3	104.6	103.2	97.1	153.4	149.5	135.8	186.4	226
228	102.2	105.6	104.2	98.1	154.8	150.8	137.0	188.0	228
230	103.2	106.6	105.2	99.1	156.2	152.2	138.2	189.7	230
232	104.1	107.6	106.2	100.1	157.6	153.6	139.4	191.3	232
234	105.1	108.6	107.2	101.1	159.0	154.9	140.7	193.0	234
236	106.0	109.5	108.2	102.1	160.3	156.3	141.9	194.7	236
238	107.0	110.5	109.2	103.1	161.7	157.6	143.2	196.3	238
240	108.0	111.5	110.1	104.0	163.1	159.0	144.4	198.0	240
242	108.9	112.5	111.1	105.0	164.5	160.3	145.6	199.7	242
244	109.9	113.5	112.1	106.0	165.9	161.7	146.9	201.3	244
246	110.8	114.5	113.1	107.0	167.3	163.1	148.1	203.0	246
248	111.8	115.4	114.1	108.0	168.7	164.4	149.3	204.7	248

44.11

*Munson and Walker's table.*—Continued.  
(Expressed in milligrams.)

CUPROUS OXIDE (Cu <sub>2</sub> O)	DEXTROSE (D-GLUCOSE)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	LACTOSE AND SUCROSE		MALTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	CUPROUS OXIDE (Cu <sub>2</sub> O)
			0.4 gram total sugar	2 grams total sugar		1 lactose, 4 sucrose	1 lactose, 12 sucrose		
250	112.8	116.4	115.1	109.0	170.1	165.8	150.6	206.3	250
252	113.7	117.4	116.1	110.0	171.5	167.2	151.8	208.0	252
254	114.7	118.4	117.1	111.0	172.8	168.5	153.1	209.7	254
256	115.7	119.4	118.1	112.0	174.2	169.9	154.3	211.3	256
258	116.6	120.4	119.1	113.0	175.6	171.3	155.5	213.0	258
260	117.6	121.4	120.1	114.0	177.0	172.6	156.8	214.7	260
262	118.6	122.4	121.1	115.0	178.4	174.0	158.0	216.3	262
264	119.5	123.4	122.1	116.0	179.8	175.3	159.3	218.0	264
266	120.5	124.4	123.1	117.0	181.2	176.7	160.5	219.7	266
268	121.5	125.4	124.1	118.0	182.6	178.1	161.8	221.3	268
270	122.5	126.4	125.1	119.0	184.0	179.4	163.0	223.0	270
272	123.4	127.4	126.2	120.0	185.3	180.8	164.3	224.6	272
274	124.4	128.4	127.2	121.1	186.7	182.2	165.5	226.3	274
276	125.4	129.4	128.2	122.1	188.1	183.5	166.8	228.0	276
278	126.4	130.4	129.2	123.1	189.5	184.9	168.0	229.6	278
280	127.3	131.4	130.2	124.1	190.9	186.3	169.3	231.3	280
282	128.3	132.4	131.2	125.1	192.3	187.6	170.5	233.0	282
284	129.3	133.4	132.2	126.1	193.7	189.0	171.8	234.6	284
286	130.3	134.4	133.2	127.1	195.1	190.4	173.0	236.3	286
288	131.3	135.4	134.3	128.1	196.5	191.7	174.3	238.0	288
290	132.3	136.4	135.3	129.2	197.8	193.1	175.5	239.6	290
292	133.2	137.4	136.3	130.2	199.2	194.4	176.8	241.3	292
294	134.2	138.4	137.3	131.2	200.6	195.8	178.1	242.9	294
296	135.2	139.4	138.3	132.2	202.0	197.2	179.3	244.6	296
298	136.2	140.5	139.4	133.2	203.4	198.6	180.6	246.3	298
300	137.2	141.5	140.4	134.2	204.8	199.9	181.8	247.9	300
302	138.2	142.5	141.4	135.3	206.2	201.3	183.1	249.6	302
304	139.2	143.5	142.4	136.3	207.6	202.7	184.4	251.3	304
306	140.2	144.5	143.4	137.3	209.0	204.0	185.6	252.9	306
308	141.2	145.5	144.5	138.3	210.4	205.4	186.9	254.6	308
310	142.2	146.6	145.5	139.4	211.8	206.8	188.1	256.3	310
312	143.2	147.6	146.5	140.4	213.2	208.1	189.4	257.9	312
314	144.2	148.6	147.6	141.4	214.6	209.5	190.7	259.6	314
316	145.2	149.6	148.6	142.4	216.0	210.9	191.9	261.2	316
318	146.2	150.7	149.6	143.5	217.3	212.2	193.2	262.9	318
320	147.2	151.7	150.7	144.5	218.7	213.6	194.4	264.6	320
322	148.2	152.7	151.7	145.5	220.1	215.0	195.7	266.2	322
324	149.2	153.7	152.7	146.6	221.5	216.4	197.0	267.9	324
326	150.2	154.8	153.8	147.6	222.9	217.7	198.2	269.6	326
328	151.2	155.8	154.8	148.6	224.3	219.1	199.5	271.2	328

*Munson and Walker's table.*—Continued.  
(Expressed in milligrams.)

CUPROUS OXIDE (Cu <sub>2</sub> O)	DEXTROROSE (d-GLUCOSE)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	LACTOSE AND SUCROSE		MALTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	CUPROUS OXIDE (Cu <sub>2</sub> O)
			0.4 gram total sugar	2 grams total sugar		1 lactose, 4 sucrose	1 lactose, 12 sucrose		
330	152.2	156.8	155.8	149.7	225.7	220.5	200.8	272.9	330
332	153.2	157.9	156.9	150.7	227.1	221.8	202.0	274.6	332
334	154.2	158.9	157.9	151.7	228.5	223.2	203.3	276.2	334
336	155.2	159.9	159.0	152.8	229.9	224.6	204.6	277.9	336
338	156.3	161.0	160.0	153.8	231.3	226.0	205.9	279.5	338
340	157.3	162.0	161.0	154.8	232.7	227.4	207.1	281.2	340
342	158.3	163.1	162.1	155.9	234.1	228.7	208.4	282.9	342
344	159.3	164.1	163.1	156.9	235.5	230.1	209.7	284.5	344
346	160.3	165.1	164.2	158.0	236.9	231.5	211.0	286.2	346
348	161.4	166.2	165.2	159.0	238.3	232.9	212.2	287.9	348
350	162.4	167.2	166.3	160.1	239.7	234.3	213.5	289.5	350
352	163.4	168.3	167.3	161.1	241.1	235.6	214.8	291.2	352
354	164.4	169.3	168.4	162.2	242.5	237.0	216.1	292.8	354
356	165.4	170.4	169.4	163.2	243.9	238.4	217.3	294.5	356
358	166.5	171.4	170.5	164.3	245.3	239.8	218.6	296.2	358
360	167.5	172.5	171.5	165.3	246.7	241.2	219.9	297.8	360
362	168.5	173.5	172.6	166.4	248.1	242.5	221.2	299.5	362
364	169.6	174.6	173.7	167.4	249.5	243.9	222.5	301.2	364
366	170.6	175.6	174.7	168.5	250.9	245.3	223.7	302.8	366
368	171.6	176.7	175.8	169.5	252.3	246.7	225.0	304.5	368
370	172.7	177.7	176.8	170.6	253.7	248.1	226.3	306.1	370
372	173.7	178.8	177.9	171.6	255.1	249.5	227.6	307.8	372
374	174.7	179.8	179.0	172.7	256.5	250.9	228.9	309.5	374
376	175.8	180.9	180.0	173.7	257.9	252.2	230.2	311.1	376
378	176.8	182.0	181.1	174.8	259.3	253.6	231.5	312.8	378
380	177.9	183.0	182.1	175.9	260.7	255.0	232.8	314.5	380
382	178.9	184.1	183.2	176.9	262.1	256.4	234.1	316.1	382
384	180.0	185.2	184.3	178.0	263.5	257.8	235.4	317.8	384
386	181.0	186.2	185.4	179.1	264.9	259.2	236.6	319.4	386
388	182.0	187.3	186.4	180.1	266.5	260.5	237.9	321.1	388
390	183.1	188.4	187.5	181.2	267.7	261.9	239.2	322.8	390
392	184.1	189.4	188.6	182.3	269.1	263.3	240.5	324.4	392
394	185.2	190.5	189.7	183.3	270.5	264.7	241.8	326.1	394
396	186.2	191.6	190.7	184.4	271.9	266.1	243.1	327.7	396
398	187.3	192.7	191.8	185.5	273.3	267.5	244.4	329.4	398
400	188.4	193.7	192.9	186.5	274.7	268.9	245.7	331.1	400
402	189.4	194.8	194.0	187.6	276.1	270.3	247.0	332.7	402
404	190.5	195.9	195.0	188.7	277.5	271.7	248.3	334.4	404
406	191.5	197.0	196.1	189.8	278.9	273.0	249.6	336.0	406
408	192.6	198.1	197.2	190.8	280.3	274.4	251.0	337.7	408

44.11

*Munson and Walker's table.*—Concluded.  
(Expressed in milligrams.)

CUPROUS OXIDE (Cu <sub>2</sub> O)	DEXTRROSE (d-GLUCOSE)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	LACTOSE AND SUCROSE		MALTOSE C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	CUPROUS OXIDE (Cu <sub>2</sub> O)
			0.4 gram total sugar	2 grains total sugar		1 lactose, 4 sucrose	1 lactose, 12 sucrose		
410	193.7	199.1	198.3	191.9	281.7	275.8	252.3	339.4	410
412	194.7	200.2	199.4	193.0	283.2	277.2	253.6	341.0	412
414	195.8	201.3	200.5	194.1	284.6	278.6	254.9	342.7	414
416	196.8	202.4	201.6	195.2	286.0	280.0	256.2	344.4	416
418	197.9	203.5	202.6	196.2	287.4	281.4	257.5	346.0	418
420	199.0	204.6	203.7	197.3	288.8	282.8	258.8	347.7	420
422	200.1	205.7	204.8	198.4	290.2	284.2	260.1	349.3	422
424	201.1	206.7	205.9	199.5	291.6	285.6	261.4	351.0	424
426	202.2	207.8	207.0	200.6	293.0	287.0	262.7	352.7	426
428	203.3	208.9	208.1	201.7	294.4	288.4	264.0	354.3	428
430	204.4	210.0	209.2	202.7	295.8	289.8	265.4	356.0	430
432	205.5	211.1	210.3	203.8	297.2	291.2	266.6	357.6	432
434	206.5	212.2	211.4	204.9	298.6	292.6	268.0	359.3	434
436	207.6	213.3	212.5	206.0	300.0	294.0	269.3	361.0	436
438	208.7	214.4	213.6	207.1	301.4	295.4	270.6	362.6	438
440	209.8	215.5	214.7	208.2	302.8	296.8	272.0	364.3	440
442	210.9	216.6	215.8	209.3	304.2	298.2	273.3	365.9	442
444	212.0	217.8	216.9	210.4	305.6	299.6	274.6	367.6	444
446	213.1	218.9	218.0	211.5	307.0	301.0	275.9	369.3	446
448	214.1	220.0	219.1	212.6	308.4	302.4	277.2	370.9	448
450	215.2	221.1	220.2	213.7	309.9	303.8	278.6	372.6	450
452	216.3	222.2	221.4	214.8	311.3	305.2	279.9	374.2	452
454	217.4	223.3	222.5	215.9	312.7	306.6	281.2	375.9	454
456	218.5	224.4	223.6	217.0	314.1	308.0	282.5	377.6	456
458	219.6	225.5	224.7	218.1	315.5	309.4	283.9	379.2	458
460	220.7	226.7	225.8	219.2	316.9	310.8	285.2	380.9	460
462	221.8	227.8	226.9	220.3	318.3	312.2	286.5	382.5	462
464	222.9	228.9	228.1	221.4	319.7	313.6	287.8	384.2	464
466	224.0	230.0	229.2	222.5	321.1	315.0	289.2	385.9	466
468	225.1	231.2	230.3	223.7	322.5	316.4	290.5	387.5	468
470	226.2	232.3	231.4	224.8	323.9	317.7	291.8	389.2	470
472	227.4	233.4	232.5	225.9	325.3	319.1	293.2	390.8	472
474	228.3	234.5	233.7	227.0	326.8	320.5	294.5	392.5	474
476	229.6	235.7	234.8	228.1	328.2	321.9	295.8	394.2	476
478	230.7	236.8	235.9	229.2	329.6	323.3	297.1	395.8	478
480	231.8	237.9	237.1	230.3	331.0	324.7	298.5	397.5	480
482	232.9	239.1	238.2	231.5	332.4	326.1	299.8	399.1	482
484	234.1	240.2	239.3	232.6	333.8	327.5	301.1	400.8	484
486	235.2	241.4	240.5	233.7	335.2	328.9	302.5	402.4	486
488	236.3	242.5	241.6	234.8	336.6	330.3	303.8	404.1	488
490	237.4	243.6	242.7	236.0	338.0	331.7	305.1	405.8	490



*Hammond's revised table for calculating dextrose, levulose, invert sugar 44.12 alone, and invert sugar in the presence of sucrose (0.3, 0.4, and 2.0 g total sugar)<sup>1</sup>*

(Applicable when Cu is determined by analysis)

(Expressed in milligrams.)

COPPER (Cu)	DEXTROSE	INVERT SUGAR	INVERT SUGAR AND SUCROSE			LEVULOSE
			0.3 g of total sugar	0.4 g of total sugar	2.0 g of total sugar	
10	4.6	5.2	3.2	2.9	.....	5.1
12	5.6	6.2	4.2	3.9	.....	6.1
14	6.5	7.2	5.3	4.9	.....	7.2
16	7.5	8.2	6.3	5.9	.....	8.3
18	8.5	9.2	7.3	6.9	.....	9.3
20	9.4	10.2	8.3	7.9	1.9	10.4
22	10.4	11.2	9.3	8.9	2.9	11.5
24	11.4	12.3	10.4	10.0	3.9	12.5
26	12.3	13.3	11.4	11.0	4.9	13.6
28	13.3	14.3	12.4	12.0	6.0	14.7
30	14.3	15.3	13.4	13.0	7.0	15.8
32	15.3	16.3	14.5	14.1	8.0	16.8
34	16.2	17.3	15.5	15.1	9.0	17.9
36	17.2	18.3	16.5	16.1	10.1	19.0
38	18.2	19.4	17.6	17.1	11.1	20.1
40	19.2	20.4	18.6	18.2	12.1	21.1
42	20.1	21.4	19.6	19.2	13.1	22.2
44	21.1	22.4	20.7	20.2	14.2	23.3
46	22.1	23.5	21.7	21.3	15.2	24.4
48	23.1	24.5	22.7	22.3	16.2	25.4
50	24.1	25.5	23.8	23.3	17.3	26.5
52	25.1	26.5	24.8	24.3	18.3	27.6
54	26.1	27.6	25.8	25.4	19.3	28.7
56	27.0	28.6	26.9	26.4	20.4	29.8
58	28.0	29.6	27.9	27.5	21.4	30.9
60	29.0	30.6	28.9	28.5	22.5	31.9
62	30.0	31.7	30.0	29.5	23.5	33.0
64	31.0	32.7	31.0	30.6	24.5	34.1
66	32.0	33.7	32.1	31.6	25.6	35.2
68	33.0	34.8	33.1	32.7	26.6	36.3
70	34.0	35.8	34.2	33.7	27.7	37.4
72	35.0	36.8	35.2	34.7	28.7	38.5
74	36.0	37.9	36.3	35.8	29.8	39.6
76	37.0	38.9	37.3	36.8	30.8	40.7
78	38.0	40.0	38.4	37.9	31.9	41.7
80	39.0	41.0	39.4	38.9	32.9	42.8
82	40.0	42.0	40.5	40.0	34.0	43.9
84	41.0	43.1	41.5	41.0	35.0	45.0
86	42.0	44.1	42.6	42.1	36.1	46.1
88	43.0	45.2	43.6	43.1	37.1	47.2
90	44.0	46.2	44.7	44.2	38.2	48.3
92	45.0	47.3	45.7	45.2	39.2	49.4
94	46.0	48.3	46.8	46.3	40.3	50.5
96	47.0	49.4	47.8	47.4	41.3	51.6
98	48.0	50.4	48.9	48.4	42.4	52.7

<sup>1</sup> L. D. Hammond, *J. Research, Nat. Bur. Standards*, 24, 589 (1940); *J. Assoc. Official Agr. Chemists* 26, 101 (1943).

44.12

*Hammond's table.*—Continued.  
(Expressed in milligrams.)

COPPER (Cu)	DEXTROSE	INVERT SUGAR	INVERT SUGAR AND SUCROSE			LEVULOSE
			0.3 g of total sugar	0.4 g of total sugar	2.0 g of total sugar	
100	49.0	51.5	50.0	49.5	43.5	53.8
102	50.0	52.5	51.0	50.5	44.5	54.9
104	51.1	53.6	52.1	51.6	45.6	56.0
106	52.1	54.6	53.1	52.7	46.7	57.1
108	53.1	55.7	54.2	53.7	47.7	58.2
110	54.1	56.7	55.3	54.8	48.8	59.3
112	55.1	57.8	56.3	55.8	49.9	60.4
114	56.1	58.9	57.4	56.9	50.9	61.6
116	57.2	59.9	58.5	58.0	52.0	62.7
118	58.2	61.0	59.5	59.0	53.1	63.8
120	59.2	62.0	60.6	60.1	54.1	64.9
122	60.2	63.1	61.7	61.2	55.2	66.0
124	61.3	64.2	62.8	62.3	56.3	67.1
126	62.3	65.2	63.8	63.3	57.4	68.2
128	63.3	66.3	64.9	64.4	58.4	69.3
130	64.3	67.4	66.0	65.5	59.5	70.4
132	65.4	68.4	67.1	66.6	60.6	71.6
134	66.4	69.5	68.1	67.6	61.7	72.7
136	67.4	70.6	69.2	68.7	62.8	73.8
138	68.5	71.6	70.3	69.8	63.9	74.9
140	69.5	72.7	71.4	70.9	64.9	76.0
142	70.5	73.8	72.5	72.0	66.0	77.1
144	71.6	74.9	73.5	73.0	67.1	78.3
146	72.6	75.9	74.6	74.1	68.2	79.4
148	73.7	77.0	75.7	75.2	69.3	80.5
150	74.7	78.1	76.8	76.3	70.4	81.6
152	75.7	79.2	77.9	77.4	71.5	82.8
154	76.8	80.3	79.0	78.5	72.6	83.9
156	77.8	81.3	80.1	79.6	73.7	85.0
158	78.9	82.4	81.2	80.6	74.8	86.1
160	79.9	83.5	82.2	81.7	75.9	87.3
162	81.0	84.6	83.3	82.8	77.0	88.4
164	82.0	85.7	84.4	83.9	78.1	89.5
166	83.1	86.8	85.5	85.0	79.2	90.6
168	84.1	87.8	86.6	86.1	80.3	91.8
170	85.2	88.9	87.7	87.2	81.4	92.9
172	86.2	90.0	88.8	88.3	82.5	94.0
174	87.3	91.1	89.9	89.4	83.6	95.2
176	88.3	92.2	91.0	90.5	84.7	96.3
178	89.4	93.3	92.1	91.6	85.8	97.4
180	90.4	94.4	93.2	92.7	86.9	98.6
182	91.5	95.5	94.3	93.8	88.0	99.7
184	92.6	96.6	95.4	94.9	89.1	100.9
186	93.6	97.7	96.5	96.0	90.2	102.0
188	94.7	98.8	97.6	97.1	91.3	103.1

*Hammond's table.*—Continued.  
(Expressed in milligrams)

44.12

COPPER (Cu)	DEXTROSE	INVERT SUGAR	INVERT SUGAR AND SUCROSE			LEVULOSE
			0.3 g of total sugar	0.4 g of total sugar	2.0 g of total sugar	
190	95.7	99.9	98.7	98.2	92.4	104.3
192	96.8	101.0	99.9	99.4	93.6	105.4
194	97.9	102.1	101.0	100.5	94.7	106.6
196	98.9	103.2	102.1	101.6	95.8	107.7
198	100.0	104.3	103.2	102.7	96.9	108.8
200	101.1	105.4	104.3	103.8	98.0	110.0
202	102.2	106.5	105.4	104.9	99.2	111.1
204	103.2	107.6	106.5	106.0	100.3	112.3
206	104.3	108.7	107.6	107.2	101.4	113.4
208	105.4	109.8	108.8	108.3	102.5	114.6
210	106.5	110.9	109.9	109.4	103.7	115.7
212	107.5	112.1	111.0	110.5	104.8	116.9
214	108.6	113.2	112.1	111.6	105.9	118.0
216	109.7	114.3	113.2	112.8	107.1	119.2
218	110.8	115.4	114.4	113.9	108.2	120.3
220	111.9	116.5	115.5	115.0	109.3	121.5
222	112.9	117.6	116.6	116.1	110.5	122.6
224	114.0	118.8	117.7	117.3	111.6	123.8
226	115.1	119.9	118.9	118.4	112.7	125.0
228	116.2	121.0	120.0	119.5	113.9	126.1
230	117.3	122.1	121.1	120.7	115.0	127.3
232	118.4	123.3	122.3	121.8	116.2	128.4
234	119.5	124.4	123.4	122.9	117.3	129.6
236	120.6	125.5	124.5	124.1	118.4	130.8
238	121.7	126.6	125.7	125.2	119.6	131.9
240	122.7	127.8	126.8	126.3	120.7	133.1
242	123.8	128.9	127.9	127.5	121.9	134.2
244	124.9	130.0	129.1	128.6	123.0	135.4
246	126.0	131.2	130.2	129.8	124.2	136.6
248	127.1	132.3	131.3	130.9	125.3	137.7
250	128.2	133.4	132.5	132.0	126.5	138.9
252	129.3	134.6	133.6	133.2	127.6	140.1
254	130.4	135.7	134.8	134.3	128.8	141.3
256	131.6	136.8	135.9	135.5	130.0	142.4
258	132.7	138.0	137.1	136.6	131.1	143.6
260	133.8	139.1	138.2	137.8	132.3	144.8
262	134.9	140.3	139.4	138.9	133.4	145.9
264	136.0	141.4	140.5	140.1	134.6	147.1
266	137.1	142.6	141.7	141.2	135.8	148.3
268	138.2	143.7	142.8	142.4	136.9	149.5
270	139.3	144.8	144.0	143.5	138.1	150.6
272	140.4	146.0	145.1	144.7	139.3	151.8
274	141.6	147.1	146.3	145.9	140.4	153.0
276	142.7	148.3	147.4	147.0	141.6	154.2
278	143.8	149.4	148.6	148.2	142.8	155.4

44.12

*Hammond's table.—Continued.*  
(Expressed in milligrams.)

COPPER (Cu)	DEXTROSE	INVERT SUGAR	INVERT SUGAR AND SUCROSE			LEVULOSE
			0.3 g of total sugar	0.4 g of total sugar	2.0 g of total sugar	
280	144.9	150.6	149.7	149.3	143.9	156.5
282	146.0	151.8	150.9	150.5	145.1	157.7
284	147.2	152.9	152.1	151.7	146.3	158.9
286	148.3	154.1	153.2	152.8	147.5	160.1
288	149.4	155.2	154.4	154.0	148.6	161.3
290	150.5	156.4	155.5	155.2	149.8	162.5
292	151.7	157.5	156.7	156.3	151.0	163.7
294	152.8	158.7	157.9	157.5	152.2	164.9
296	153.9	159.9	159.0	158.7	153.4	166.0
298	155.1	161.0	160.2	159.9	154.6	167.2
300	156.2	162.2	161.4	161.0	155.7	168.4
302	157.3	163.4	162.5	162.2	156.9	169.6
304	158.5	164.5	163.7	163.4	158.1	170.8
306	159.6	165.7	164.9	164.6	159.3	172.0
308	160.7	166.9	166.1	165.7	160.5	173.2
310	161.9	168.0	167.2	166.9	161.7	174.4
312	163.0	169.2	168.4	168.1	162.9	175.6
314	164.2	170.4	169.6	169.3	164.1	176.8
316	165.3	171.6	170.8	170.5	165.3	178.0
318	166.5	172.8	172.0	171.7	166.5	179.2
320	167.6	173.9	173.1	172.8	167.7	180.4
322	168.8	175.1	174.3	174.0	168.9	181.6
324	169.9	176.3	175.5	175.2	170.1	182.8
326	171.1	177.5	176.7	176.4	171.3	184.0
328	172.2	178.7	177.9	177.6	172.5	185.2
330	173.4	179.8	179.1	178.8	173.7	186.4
332	174.5	181.0	180.3	180.0	174.9	187.6
334	175.7	182.2	181.5	181.2	176.1	188.8
336	176.8	183.4	182.6	182.4	177.3	190.1
338	178.0	184.6	183.8	183.6	178.6	191.3
340	179.2	185.8	185.0	184.8	179.8	192.5
342	180.3	187.0	186.2	186.0	181.0	193.7
344	181.5	188.2	187.4	187.2	182.2	194.9
346	182.7	189.4	188.6	188.4	183.4	196.1
348	183.8	190.6	189.8	189.6	184.6	197.3
350	185.0	191.8	191.0	190.8	185.9	198.5
352	186.2	193.0	192.2	192.0	187.1	199.8
354	187.3	194.2	193.4	193.2	188.3	201.0
356	188.5	195.4	194.6	194.4	189.5	202.2
358	189.7	196.6	195.8	195.7	190.8	203.4
360	190.9	197.8	197.1	196.9	192.0	204.7
362	192.0	199.0	198.3	198.1	193.2	205.9
364	193.2	200.2	199.5	199.3	194.5	207.1
366	194.4	201.4	200.7	200.5	195.7	208.3
368	195.6	202.6	201.9	201.7	196.9	209.6

*Hammond's table.*—Concluded.  
(Expressed in milligrams.)

COPPER (Cu)	DEXTROSE	INVERT SUGAR	INVERT SUGAR AND SUCROSE			LEVULOSE
			0.3 g of total sugar	0.4 g of total sugar	2.0 g of total sugar	
370	196.8	203.8	203.1	203.0	198.2	210.8
372	198.0	205.0	204.3	204.2	199.4	212.0
374	199.1	206.3	205.6	205.4	200.7	213.3
376	200.3	207.5	206.8	206.6	201.9	214.5
378	201.5	208.7	208.0	207.9	203.1	215.7
380	202.7	209.9	209.2	209.1	204.4	217.0
382	203.9	211.1	210.4	210.3	205.6	218.2
384	205.1	212.4	211.7	211.6	206.9	219.5
386	206.3	213.6	212.9	212.8	208.1	220.7
388	207.5	214.8	214.1	214.0	209.4	221.9
390	208.7	216.0	215.4	215.3	210.6	223.2
392	209.9	217.3	216.6	216.5	211.9	224.4
394	211.1	218.5	217.8	217.8	213.2	225.7
396	212.3	219.8	219.1	219.0	214.4	226.9
398	213.5	221.0	220.3	220.3	215.7	228.2
400	214.7	222.2	221.5	221.5	217.0	229.4
402	215.9	223.5	222.8	222.8	218.2	230.7
404	217.1	224.7	224.0	224.0	219.5	232.0
406	218.4	226.0	225.3	225.3	220.8	233.2
408	219.6	227.2	226.6	226.5	222.0	234.5
410	220.8	228.5	227.8	227.8	223.3	235.8
412	222.0	229.7	229.1	229.1	224.6	237.1
414	223.3	231.0	230.4	230.4	225.9	238.4
416	224.5	232.3	231.6	231.7	227.2	239.7
418	225.7	233.6	232.9	232.9	228.5	241.0
420	227.0	234.8	234.2	234.2	229.8	242.2
422	228.2	236.1	235.5	235.5	231.1	243.6
424	229.5	237.5	236.8	236.9	232.4	244.9
426	230.7	238.8	238.2	238.2	233.8	246.3
428	232.0	240.2	239.5	239.6	235.1	247.8
430	233.3	241.5	240.9	241.0	236.5	249.2
432	234.7	243.0	242.4	242.5	238.0	250.8
434	236.1	244.7	244.1	244.2	239.6	252.7

44.13

*Herzfeld's table for determining invert sugar in raw sugars  
(invert sugar not to exceed 1.5 per cent).<sup>1</sup>*

COPPER (Cu)	INVERT SUGAR	COPPER (Cu)	INVERT SUGAR	COPPER (Cu)	INVERT SUGAR	COPPER (Cu)	INVERT SUGAR	COPPER (Cu)	INVERT SUGAR
mg.	per cent	mg.	per cent	mg.	per cent	mg.	per cent	mg.	per cent
50	0.050	110	0.351	170	0.680	230	1.013	290	1.357
52	.058	112	.361	172	.692	232	.024	292	.368
54	.066	114	.371	174	.704	234	.036	294	.380
56	.074	116	.381	176	.715	236	.047	296	.391
58	.082	118	.392	178	.726	238	.058	298	.403
60	.090	120	.402	180	.737	240	.070	300	.414
62	.098	122	.412	182	.748	242	.081	302	.425
64	.108	124	.423	184	.759	244	.093	304	.437
66	.118	126	.433	186	.770	246	.104	306	.448
68	.128	128	.443	188	.781	248	.116	308	.460
70	.138	130	.453	190	.792	250	.127	310	.471
72	.148	132	.463	192	.803	252	.139	312	.483
74	.157	134	.473	194	.814	254	.150	314	.494
76	.167	136	.483	196	.825	256	.162		
78	.177	138	.493	198	.836	258	.173		
80	.187	140	.503	200	.847	260	.185		
82	.197	142	.515	202	.858	262	.196		
84	.208	144	.527	204	.869	264	.207		
86	.219	146	.538	206	.880	266	.219		
88	.231	148	.550	208	.891	268	.231		
90	.242	150	.562	210	.902	270	.242		
92	.254	152	.574	212	.913	272	.253		
94	.265	154	.586	214	.924	274	.265		
96	.277	156	.598	216	.935	276	.276		
98	.288	158	.609	218	.946	278	.288		
100	.300	160	.621	220	.957	280	.299		
102	.310	162	.633	222	.968	282	.311		
104	.320	164	.645	224	.979	284	.322		
106	.330	166	.657	226	.990	286	.334		
108	.340	168	.669	228	1.001	288	.345		

<sup>1</sup> *Z. Ver. Ruhebzucker-Ind.*, 35 (N.F. 22); 1012 (1885).

*Corrections to be applied to iodine titer to obtain  
milligrams of invert sugar by Ofner method.<sup>1</sup>*

44.14

0.0323 N IODINE SOLN (ML)	WEIGHT OF SUCROSE (GRAMS)									
	1	2	3	4	5	6	7	8	9	10
1	0.11	0.22	0.34	0.45	0.55	0.66	0.77	0.89	1.00	1.11
2	.17	.28	.40	.51	.61	.72	.84	.95	1.06	1.16
3	.22	.34	.45	.57	.67	.78	.90	1.01	1.12	1.22
4	.28	.39	.51	.62	.73	.84	.95	1.07	1.18	1.28
5	.33	.45	.56	.68	.78	.90	1.01	1.12	1.24	1.33
6	.39	.50	.61	.73	.83	.95	1.06	1.18	1.29	1.39
7	.44	.55	.67	.78	.88	1.00	1.11	1.23	1.34	1.44
8	.49	.60	.72	.83	.94	1.05	1.16	1.28	1.39	1.50
9	.54	.65	.76	.88	.99	1.10	1.21	1.33	1.44	1.55
10	.59	.70	.82	.93	1.03	1.15	1.26	1.37	1.49	1.60
11	.63	.75	.86	.98	1.08	1.20	1.31	1.42	1.54	1.65
12	.67	.78	.90	1.02	1.12	1.24	1.35	1.47	1.58	1.69
13	.70	.82	.93	1.05	1.16	1.27	1.39	1.51	1.62	1.72
14	.74	.85	.97	1.09	1.19	1.31	1.42	1.54	1.65	1.76
15	.77	.88	1.00	1.12	1.22	1.34	1.45	1.57	1.69	1.79
16	.80	.91	1.03	1.15	1.25	1.37	1.48	1.60	1.72	1.82
17	.82	.94	1.05	1.18	1.28	1.40	1.51	1.63	1.74	1.85
18	.84	.96	1.08	1.20	1.30	1.42	1.54	1.66	1.77	1.88
19	.86	.98	1.10	1.22	1.32	1.45	1.56	1.68	1.79	1.90
20	.88	1.00	1.11	1.24	1.34	1.46	1.58	1.70	1.81	1.92
21	.89	1.01	1.13	1.25	1.35	1.48	1.59	1.71	1.83	1.94
22	.86	.98	1.11	1.23	1.34	1.47	1.59	1.71	1.84	1.95

<sup>1</sup> J. Assoc. Official Agr. Chem., 26, 470 (1943).

*Meissl and Hiller's factors for determining invert sugar in materials in which, of the total sugars present, more than 1.5 per cent is invert sugar, and less than 98.5 per cent is sucrose.<sup>1</sup>*

RATIO OF SUCROSE TO INVERT SUGAR = R:1	APPROXIMATE ABSOLUTE WEIGHT OF INVERT SUGAR (Z)						
	200 mg	175 mg	150 mg	125 mg	100 mg	75 mg	50 mg
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
0:100	56.4	55.4	54.5	53.8	53.2	53.0	53.0
10:90	56.3	55.3	54.4	53.8	53.2	52.9	52.9
20:80	56.2	55.2	54.3	53.7	53.2	52.7	52.7
30:70	56.1	55.1	54.2	53.7	53.2	52.6	52.6
40:60	55.9	55.0	54.1	53.6	53.1	52.5	52.4
50:50	55.7	54.9	54.0	53.5	53.1	52.3	52.2
60:40	55.6	54.7	53.8	53.2	52.8	52.1	51.9
70:30	55.5	54.5	53.5	52.9	52.5	51.9	51.6
80:20	55.4	54.3	53.3	52.7	52.2	51.7	51.3
90:10	54.6	53.6	53.1	52.6	52.1	51.6	51.2
91:9	54.1	53.6	52.6	52.1	51.6	51.2	50.7
92:8	53.6	53.1	52.1	51.6	51.2	50.7	50.3
93:7	53.6	53.1	52.1	51.2	50.7	50.3	49.8
94:6	53.1	52.6	51.6	50.7	50.3	49.8	48.9
95:5	52.6	52.1	51.2	50.3	49.4	48.9	48.5
96:4	52.1	51.2	50.7	49.8	48.9	47.7	46.9
97:3	50.7	50.3	49.8	48.9	47.7	46.2	45.1
98:2	49.9	48.9	48.5	47.3	45.8	43.3	40.0
99:1	47.7	47.3	46.5	45.1	43.3	41.2	38.1

<sup>1</sup> Z. Ver. Rübensucker-Ind., 39 (N.F. 26) 734 (1889).

44.16

Wein's table for the determination of maltose.<sup>1</sup>  
(Expressed in milligrams.)

COPPER	CUPROUS OXIDE	MALTOSE	COPPER	CUPROUS OXIDE*	MALTOSE	COPPER	CUPROUS OXIDE	MALTOSE
32	36.0	27.0	122	137.4	106.2	212	238.7	186.8
34	38.3	28.7	124	139.6	108.0	214	240.9	188.6
36	40.5	30.5	126	141.9	109.8	216	243.2	190.4
38	42.8	32.2	128	144.1	111.6	218	245.4	192.1
40	45.0	33.9	130	146.4	113.4	220	247.7	193.9
42	47.3	35.7	132	148.6	115.2	222	249.9	195.7
44	49.5	37.4	134	150.9	117.0	224	252.4	197.5
46	51.8	39.1	136	153.1	118.8	226	254.4	199.3
48	54.0	40.9	138	155.4	120.6	228	256.7	201.1
50	56.3	42.6	140	157.6	122.4	230	258.9	202.9
52	58.5	44.4	142	159.9	124.2	232	261.2	204.7
54	60.8	46.1	144	162.1	126.0	234	263.4	206.5
56	63.0	47.8	146	164.4	127.8	236	265.7	208.3
58	65.3	49.6	148	166.6	129.6	238	268.0	210.0
60	67.6	51.3	150	168.9	131.4	240	270.2	211.8
62	69.8	53.1	152	171.1	133.2	242	272.5	213.6
64	72.1	54.8	154	173.4	135.0	244	274.7	215.4
66	74.3	56.6	156	175.6	136.8	246	277.0	217.2
68	76.6	58.3	158	177.9	138.6	248	279.2	219.0
70	78.8	60.1	160	180.1	140.4	250	281.5	220.8
72	81.1	61.8	162	182.4	142.2	252	283.7	222.6
74	83.3	63.6	164	184.6	144.0	254	286.0	224.4
76	85.6	65.4	166	186.9	145.8	256	288.2	226.2
78	87.8	67.1	168	189.1	147.8	258	290.5	228.0
80	90.1	68.9	170	191.4	149.4	260	292.7	229.8
82	92.3	70.6	172	193.6	151.2	262	295.0	231.6
84	94.6	72.4	174	195.9	152.9	264	297.2	233.4
86	96.8	74.1	176	198.1	154.7	266	299.5	235.2
88	99.1	75.9	178	200.4	156.5	268	301.7	237.0
90	101.3	77.7	180	202.6	158.3	270	304.0	238.8
92	103.6	79.5	182	204.9	160.1	272	306.2	240.6
94	105.8	81.2	184	207.1	161.8	274	308.5	242.4
96	108.1	83.0	186	209.4	163.6	276	310.7	244.2
98	110.3	84.8	188	211.7	165.4	278	313.0	246.0
100	112.6	86.6	190	213.9	167.2	280	315.2	247.8
102	114.8	88.4	192	216.2	169.0	282	317.5	249.6
104	117.1	90.1	194	218.4	170.7	284	319.7	251.3
106	119.3	91.9	196	220.7	172.5	286	322.0	253.1
108	121.6	93.7	198	222.9	174.3	288	324.2	254.9
110	123.8	95.5	200	225.2	176.1	290	326.5	256.6
112	126.1	97.3	202	227.4	177.9	292	328.7	258.4
114	128.3	99.0	204	229.7	179.6	294	331.0	260.2
116	130.6	100.8	206	231.9	181.4	296	333.2	262.0
118	132.8	102.6	208	234.2	183.2	298	335.5	263.7
120	135.1	104.4	210	236.4	185.0	300	337.8	265.5

<sup>1</sup> Tables for the Quantitative Estimation of the Sugars. Translated by Frew, 1896, p. 26.



*Copper-levulose equivalents according to Jackson and Mathews' modification of Nyns' selective method for levulose.*

44.17

(Expressed in milligrams. A linear interpolation yields accurate results.)

Cu	LEVULOSE	Cu	LEVULOSE
5	2.5	130	39.3
10	4.5	140	42.0
15	6.2	150	44.7
20	7.9	160	47.4
25	9.5	170	50.0
30	11.0	180	52.6
35	12.5	190	55.2
40	13.9	200	57.9
45	15.4	210	60.6
50	16.8	220	63.4
55	18.3	230	66.4
60	19.7	240	69.4
65	21.2	250	72.5
70	22.5	260	75.7
80	25.4	270	79.0
90	28.1	280	82.4
100	30.9	290	85.9
110	33.7	300	89.5
120	36.5	310	93.2

44.18

*Allihn's table for the determination of dextrose.<sup>1</sup>*

(Expressed in milligrams.)

COPPER	DEXTROSE	COPPER	DEXTROSE	COPPER	DEXTROSE
12	7.1	102	51.9	192	98.4
14	8.1	104	52.9	194	99.4
16	9.0	106	54.0	196	100.5
18	10.0	108	55.0	198	101.5
20	11.0	110	56.0	200	102.6
22	12.0	112	57.0	202	103.7
24	13.0	114	58.0	204	104.7
26	14.0	116	59.1	206	105.8
28	15.0	118	60.1	208	106.8
30	16.0	120	61.1	210	107.9
32	17.0	122	62.1	212	109.0
34	18.0	124	63.1	214	110.0
36	18.9	126	64.2	216	111.1
38	19.9	128	65.2	218	112.1
40	20.9	130	66.2	220	113.2
42	21.9	132	67.2	222	114.3
44	22.9	134	68.2	224	115.3
46	23.9	136	69.3	226	116.4
48	24.9	138	70.3	228	117.4
50	25.9	140	71.3	230	118.5
52	26.9	142	72.3	232	119.6
54	27.9	144	73.4	234	120.7
56	28.8	146	74.4	236	121.7
58	29.8	148	75.5	238	122.8
60	30.8	150	76.5	240	123.9
62	31.8	152	77.5	242	125.0
64	32.8	154	78.6	244	126.0
66	33.8	156	79.6	246	127.1
68	34.8	158	80.7	248	128.1
70	35.8	160	81.7	250	129.2
72	36.8	162	82.7	252	130.3
74	37.8	164	83.8	254	131.4
76	38.8	166	84.8	256	132.4
78	39.8	168	85.9	258	133.5
80	40.8	170	86.9	260	134.6
82	41.8	172	87.9	262	135.7
84	42.8	174	89.0	264	136.8
86	43.9	176	90.0	266	137.8
88	44.9	178	91.1	268	138.9
90	45.9	180	92.1	270	140.0
92	46.9	182	93.1	272	141.1
94	47.9	184	94.2	274	142.2
96	48.9	186	95.2	276	143.3
98	49.9	188	96.3	278	144.4
100	50.9	190	97.3	280	145.5

<sup>1</sup> Z. Ver. Rucbenzucker-Ind., 32 (N.F. 19), 606, 865 (1882).

*Allihn's table.*—Concluded.

44.18

(Expressed in milligrams.)

COPPER	DEXTROSE	COPPER	DEXTROSE	COPPER	DEXTROSE
282	146.6	342	179.8	402	214.1
284	147.7	344	180.9	404	215.2
286	148.8	346	182.1	406	216.4
288	149.9	348	183.2	408	217.5
290	151.0	350	184.3	410	218.7
292	152.1	352	185.4	412	219.9
294	153.2	354	186.6	414	221.0
296	154.3	356	187.7	416	222.2
298	155.4	358	188.9	418	223.3
300	156.5	360	190.0	420	224.5
302	157.6	362	191.1	422	225.7
304	158.7	364	192.3	424	226.9
306	159.8	366	193.4	426	228.0
308	160.9	368	194.6	428	229.2
310	162.0	370	195.7	430	230.4
312	163.1	372	196.8	432	231.6
314	164.2	374	198.0	434	232.8
316	165.3	376	199.1	436	233.9
318	166.4	378	200.3	438	235.1
320	167.5	380	201.4	440	236.3
322	168.6	382	202.5	442	237.5
324	169.7	384	203.7	444	238.7
326	170.9	386	204.8	446	239.8
328	172.0	388	206.0	448	241.0
330	173.1	390	207.1	450	242.2
332	174.2	392	208.3	452	243.4
334	175.3	394	209.4	454	244.6
336	176.5	396	210.6	456	245.7
338	177.6	398	211.7	458	246.9
340	178.7	400	212.9	460	248.1
				462	249.3

44.19 Factors for 10 ml Soxhlet solution to be used in connection with the Lane-Eynon general volumetric method.

TITER	INVERT SUGAR NO SUCROSE	1 GRAM SUCROSE PER 100 ML INVERT SUGAR	5 GRAMS SUCROSE PER 100 ML INVERT SUGAR	10 GRAMS SUCROSE PER 100 ML INVERT SUGAR	25 GRAMS SUCROSE PER 100 ML INVERT SUGAR	DEXTROSE	LEVULOSE	ANHYDROUS MALTOSE $C_{12}H_{22}O_{11}$	HYDRATED MALTOSE $C_{12}H_{22}O_{11} \cdot H_2O$	ANHYDROUS LACTOSE $C_{12}H_{22}O_{11}$	HYDRATED LACTOSE $C_{12}H_{22}O_{11} \cdot H_2O$
15	50.5	49.9	47.6	46.1	43.4	49.1	52.2	77.2	81.3	64.9	68.3
16	50.6	50.0	47.6	46.1	43.4	49.2	52.3	77.1	81.2	64.8	68.2
17	50.7	50.1	47.6	46.1	43.4	49.3	52.3	77.0	81.1	64.8	68.2
18	50.8	50.1	47.6	46.1	43.3	49.3	52.4	77.0	81.0	64.7	68.1
19	50.8	50.2	47.6	46.1	43.3	49.4	52.5	76.9	80.9	64.7	68.1
20	50.9	50.2	47.6	46.1	43.2	49.5	52.5	76.8	80.8	64.6	68.0
21	51.0	50.2	47.6	46.1	43.2	49.5	52.6	76.7	80.7	64.6	68.0
22	51.0	50.3	47.6	46.1	43.1	49.6	52.7	76.6	80.6	64.6	68.0
23	51.1	50.3	47.6	46.1	43.0	49.7	52.7	76.5	80.5	64.5	67.9
24	51.2	50.3	47.6	46.1	42.9	49.8	52.8	76.4	80.4	64.5	67.9
25	51.2	50.4	47.6	46.0	42.8	49.8	52.8	76.4	80.4	64.5	67.9
26	51.3	50.4	47.6	46.0	42.8	49.9	52.9	76.3	80.3	64.5	67.9
27	51.4	50.4	47.6	46.0	42.7	49.9	52.9	76.2	80.2	64.4	67.8
28	51.4	50.5	47.7	46.0	42.7	50.0	53.0	76.1	80.1	64.4	67.8
29	51.5	50.5	47.7	46.0	42.6	50.0	53.1	76.0	80.0	64.4	67.8
30	51.5	50.5	47.7	46.0	42.5	50.1	53.2	76.0	80.0	64.4	67.8
31	51.6	50.6	47.7	45.9	42.5	50.2	53.2	75.9	79.9	64.4	67.8
32	51.6	50.6	47.7	45.9	42.4	50.2	53.3	75.9	79.9	64.4	67.8
33	51.7	50.6	47.7	45.9	42.3	50.3	53.3	75.8	79.8	64.4	67.8
34	51.7	50.6	47.7	45.8	42.2	50.3	53.4	75.8	79.8	64.4	67.9
35	51.8	50.7	47.7	45.8	42.2	50.4	53.4	75.7	79.7	64.5	67.9
36	51.8	50.7	47.7	45.8	42.1	50.4	53.5	75.6	79.6	64.5	67.9
37	51.9	50.7	47.7	45.7	42.0	50.5	53.5	75.6	79.6	64.5	67.9
38	51.9	50.7	47.7	45.7	42.0	50.5	53.6	75.5	79.5	64.5	67.9
39	52.0	50.8	47.7	45.7	41.9	50.6	53.6	75.5	79.5	64.5	67.9
40	52.0	50.8	47.7	45.6	41.8	50.6	53.6	75.4	79.4	64.5	67.9
41	52.1	50.8	47.7	45.6	41.8	50.7	53.7	75.4	79.4	64.6	68.0
42	52.1	50.8	47.7	45.6	41.7	50.7	53.7	75.3	79.3	64.6	68.0
43	52.2	50.8	47.7	45.5	41.6	50.8	53.8	75.3	79.3	64.6	68.0
44	52.2	50.9	47.7	45.5	41.5	50.8	53.8	75.2	79.2	64.6	68.0
45	52.3	50.9	47.7	45.4	41.4	50.9	53.9	75.2	79.2	64.7	68.1
46	52.3	50.9	47.7	45.4	41.4	50.9	53.9	75.1	79.1	64.7	68.1
47	52.4	50.9	47.7	45.3	41.3	51.0	53.9	75.1	79.1	64.8	68.2
48	52.4	50.9	47.7	45.3	41.2	51.0	54.0	75.1	79.1	64.8	68.2
49	52.5	51.0	47.7	45.2	41.1	51.0	54.0	75.0	79.0	64.8	68.2
50	52.5	51.0	47.7	45.2	41.0	51.1	54.0	75.0	79.0	64.9	68.3

44.20 Factors for 25 ml Soxhlet's solution to be used in connection with the Lane-Eynon general volumetric method.

TITRE	INVERT SUGAR NO SUCROSE	1 GRAM SUCROSE PER 100 ML INVERT SUGAR	DEXTROSE	LEVULOSE	ANHYDROUS MALTOSE $C_{12}H_{22}O_{11}$	HYDRATED MALTOSE $C_{12}H_{22}O_{11} \cdot H_2O$	ANHYDROUS LACTOSE $C_{12}H_{22}O_{11}$	HYDRATED LACTOSE $C_{12}H_{22}O_{11} \cdot H_2O$
15	123.6	122.6	120.2	127.4	197.8	208.2	163.9	172.5
16	123.6	122.7	120.2	127.4	197.4	207.8	163.5	172.1
17	123.6	122.7	120.2	127.5	197.0	207.4	163.1	171.7
18	123.7	122.7	120.2	127.5	196.7	207.1	162.8	171.4
19	123.7	122.8	120.3	127.6	196.5	206.8	162.5	171.1
20	123.8	122.8	120.3	127.6	196.2	206.5	162.3	170.9
21	123.8	122.8	120.3	127.7	195.8	206.1	162.0	170.6
22	123.9	122.9	120.4	127.7	195.5	205.8	161.8	170.4
23	123.9	122.9	120.4	127.8	195.1	205.4	161.6	170.2
24	124.0	122.9	120.5	127.8	194.8	205.1	161.5	170.0
25	124.0	123.0	120.5	127.9	194.5	204.8	161.4	169.9
26	124.1	123.0	120.6	127.9	194.2	204.4	161.2	169.7
27	124.1	123.0	120.6	128.0	193.9	204.1	161.0	169.5
28	124.2	123.1	120.7	128.0	193.6	203.8	160.8	169.3
29	124.2	123.1	120.7	128.1	193.3	203.5	160.7	169.2
30	124.3	123.1	120.8	128.1	193.0	203.2	160.6	169.0
31	124.3	123.2	120.8	128.1	192.8	202.9	160.5	168.9
32	124.4	123.2	120.8	128.2	192.5	202.6	160.4	168.8
33	124.4	123.2	120.9	128.2	192.2	202.3	160.2	168.6
34	124.5	123.3	120.9	128.3	191.9	202.0	160.1	168.5
35	124.5	123.3	121.0	128.3	191.7	201.8	160.0	168.4
36	124.6	123.3	121.0	128.4	191.4	201.5	159.8	168.2
37	124.6	123.4	121.1	128.4	191.2	201.2	159.7	168.1
38	124.7	123.4	121.2	128.5	191.0	201.0	159.6	168.0
39	124.7	123.4	121.2	128.5	190.8	200.8	159.5	167.9
40	124.8	123.4	121.2	128.6	190.5	200.5	159.4	167.8
41	124.8	123.5	121.3	128.6	190.3	200.3	159.3	167.7
42	124.9	123.5	121.4	128.6	190.1	200.1	159.2	167.6
43	124.9	123.5	121.4	128.7	189.8	199.8	159.2	167.6
44	125.0	123.6	121.5	128.7	189.6	199.6	159.1	167.5
45	125.0	123.6	121.5	128.8	189.4	199.4	159.0	167.4
46	125.1	123.6	121.6	128.8	189.2	199.2	159.0	167.4
47	125.1	123.7	121.6	128.9	189.0	199.0	158.9	167.3
48	125.2	123.7	121.7	128.9	188.9	198.9	158.8	167.2
49	125.2	123.7	121.7	129.0	188.8	198.7	158.8	167.2
50	125.3	123.8	121.8	129.0	188.7	198.6	158.7	167.1

44.21 *Quisumbing and Thomas table for calculating dextrose, levulose, invert sugar, lactose, and mallose.*  
(Expressed in milligrams.)

COFFEE (C <sub>6</sub> H <sub>6</sub> O <sub>2</sub> )	CUPREOUS OXIDE (Cu <sub>2</sub> O)	DEXTROSE (β-D-Glucose)	LEVULOSE (β-FRUCTOSE)	INVERT SUGAR	LACTOSE		MALTOSE	
					C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> · H <sub>2</sub> O	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> · H <sub>2</sub> O
10	11.1	4.8	5.3	5.0	7.7	8.1	9.4	9.9
20	22.5	9.5	10.5	10.1	15.5	16.3	18.8	19.8
30	33.8	14.3	15.8	15.2	23.2	24.4	28.2	29.7
40	45.0	19.1	21.2	20.3	30.9	32.5	37.6	39.6
50	56.3	24.0	26.5	25.4	38.7	40.7	47.0	49.5
60	67.6	28.9	31.9	30.6	46.4	48.8	56.4	59.4
70	78.8	33.7	37.2	35.7	54.0	56.9	65.8	69.3
80	90.1	38.7	42.6	40.9	61.7	65.0	75.2	79.2
90	101.3	43.6	48.0	46.1	69.5	73.2	84.6	89.1
100	112.6	48.6	53.4	51.3	77.2	81.3	94.0	99.0
110	123.8	53.5	58.8	56.5	85.0	89.5	103.4	108.9
120	135.1	58.5	64.3	61.8	92.7	97.0	112.8	118.8
130	146.4	63.5	69.7	67.0	100.4	105.7	122.2	128.7
140	157.6	68.6	75.2	72.3	108.2	113.9	131.6	138.6
150	168.9	73.7	80.7	77.6	116.0	122.0	141.0	148.5
160	180.1	78.8	86.2	82.9	123.7	130.1	150.4	158.4
170	191.4	83.9	91.7	88.3	131.4	138.3	159.8	168.3
180	202.6	89.1	97.2	93.7	139.1	146.4	169.2	178.2
190	213.9	94.2	102.8	99.1	146.9	154.6	178.8	188.1
200	225.2	99.4	108.4	104.4	154.6	162.7	188.2	198.0
210	236.4	104.6	114.0	109.8	162.3	170.9	197.6	207.9
220	247.7	109.9	119.6	115.2	170.0	179.0	207.0	217.8
230	258.9	115.1	125.2	120.6	177.8	187.2	216.4	227.7
240	270.2	120.4	130.8	126.1	185.5	195.3	225.8	237.6
250	281.5	125.7	136.4	131.6	193.2	203.4	235.2	247.5
260	292.7	131.0	142.1	137.1	201.0	211.6	244.6	257.4
270	304.0	136.4	147.8	142.6	208.8	219.8	254.0	267.3
280	315.2	141.7	153.5	148.2	216.5	227.9	263.4	277.2
290	326.5	147.1	159.2	153.7	224.2	236.0	272.8	287.1
300	337.8	152.6	165.0	159.3	232.0	244.2	282.2	297.0
310	349.0	158.0	170.7	164.9	239.7	252.3	291.6	306.9
320	360.3	163.5	176.5	170.5	247.5	260.5	301.0	316.8
330	371.5	168.9	182.3	176.1	255.3	268.7	310.4	326.7
340	382.8	174.5	188.1	181.8	263.0	276.8	319.8	336.6
350	394.0	180.0	193.9	187.4	270.7	285.0	329.2	346.5
360	405.3	185.5	199.7	193.1	278.4	293.1	338.6	356.4
370	416.6	191.1	205.5	198.8	286.2	301.3	348.0	366.3
380	427.8	196.7	211.4	204.5	293.9	309.4	357.4	376.2
390	439.1	202.3	217.3	210.2	301.6	317.5	366.8	386.1
400	450.3	208.0	223.2	216.0	309.4	325.7	376.2	396.0
410	461.6	213.7	229.1	221.8	317.1	333.8	385.6	405.9
420	472.9	219.4	235.0	227.6	324.9	342.0	395.0	415.8
430	484.1	225.1	240.9	233.4	332.6	350.1	404.4	425.7
440	495.4	230.8	246.9	239.2	340.4	358.3	413.8	435.6
450	506.6	236.6	252.9	245.0	348.1	366.4	423.2	445.5
460	517.9	242.4	258.9	250.9	355.9	374.6	432.6	455.4
470	529.1	248.1	264.9	256.8	363.6	382.7	442.0	465.3
480	540.4	254.8	270.9	262.7	371.3	390.9	451.4	475.2

*Krüber's table for the determination of pentoses and pentosans.*  
(expressed in grams.)

44.22

FURFURAL PHENOLGLUCIDE	FURFURAL	ARABINOSE	ARABAN	XYLOSE	XYLAN	PENTOSE	PENTOSAN
0.030	0.0182	0.0391	0.0344	0.0324	0.0285	0.0358	0.0315
.032	.0193	.0413	.0363	.0342	.0301	.0378	.0333
.034	.0203	.0435	.0383	.0361	.0317	.0398	.0350
.036	.0214	.0457	.0402	.0379	.0334	.0418	.0368
.038	.0224	.0479	.0422	.0398	.0350	.0439	.0386
.040	.0235	.0501	.0441	.0416	.0366	.0459	.0404
.042	.0245	.0523	.0460	.0434	.0382	.0479	.0422
.044	.0255	.0545	.0480	.0452	.0398	.0499	.0440
.046	.0266	.0567	.0499	.0471	.0414	.0519	.0457
.048	.0276	.0589	.0519	.0489	.0430	.0539	.0475
.050	.0286	.0611	.0538	.0507	.0446	.0559	.0492
.052	.0297	.0633	.0557	.0525	.0462	.0579	.0510
.054	.0307	.0655	.0576	.0543	.0478	.0599	.0528
.056	.0318	.0677	.0596	.0562	.0494	.0620	.0546
.058	.0328	.0699	.0615	.0580	.0510	.0640	.0564
.060	.0338	.0721	.0634	.0598	.0526	.0660	.0581
.062	.0349	.0743	.0653	.0616	.0542	.0680	.0599
.064	.0359	.0765	.0673	.0635	.0558	.0700	.0617
.066	.0370	.0787	.0692	.0653	.0575	.0720	.0634
.068	.0380	.0809	.0712	.0672	.0591	.0741	.0652
.070	.0390	.0831	.0731	.0690	.0607	.0761	.0670
.072	.0401	.0853	.0750	.0708	.0623	.0781	.0688
.074	.0411	.0875	.0770	.0726	.0639	.0801	.0706
.076	.0422	.0897	.0789	.0745	.0655	.0821	.0722
.078	.0432	.0919	.0809	.0763	.0671	.0841	.0740
.080	.0442	.0941	.0828	.0781	.0687	.0861	.0758
.082	.0453	.0963	.0847	.0799	.0703	.0881	.0776
.084	.0463	.0985	.0867	.0817	.0719	.0901	.0794
.086	.0474	.1007	.0886	.0836	.0735	.0922	.0812
.088	.0484	.1029	.0906	.0854	.0751	.0942	.0830
.090	.0494	.1051	.0925	.0872	.0767	.0962	.0847
.092	.0505	.1073	.0944	.0890	.0783	.0982	.0865
.094	.0515	.1095	.0964	.0909	.0800	.1002	.0883
.096	.0525	.1117	.0983	.0927	.0816	.1022	.0899
.098	.0536	.1139	.1003	.0946	.0832	.1043	.0917
.100	.0546	.1161	.1022	.0964	.0848	.1063	.0935
.102	.0557	.1182	.1041	.0982	.0864	.1083	.0953
.104	.0567	.1204	.1060	.1000	.0880	.1103	.0971
.106	.0577	.1226	.1080	.1019	.0896	.1123	.0988
.108	.0588	.1248	.1099	.1037	.0912	.1143	.1006
.110	.0598	.1270	.1118	.1055	.0928	.1163	.1023
.112	.0608	.1292	.1137	.1073	.0944	.1183	.1041
.114	.0619	.1314	.1156	.1091	.0960	.1203	.1059
.116	.0629	.1336	.1176	.1110	.0976	.1223	.1076
.118	.0640	.1358	.1195	.1128	.0992	.1243	.1094

44.22

*Kröber's table.*—Continued.

(Expressed in grams.)

FURFURAL PHLOROGLUCIDE	FURFURAL	ARABINOSE	ARABAN	XYLOSE	XYLAN	PENTOSE	PENTOSAN
0.120	0.0650	0.1380	0.1214	0.1146	0.1008	0.1263	0.1111
.122	.0660	.1402	.1233	.1164	.1024	.1283	.1129
.124	.0671	.1424	.1253	.1182	.1040	.1303	.1147
.126	.0681	.1446	.1272	.1201	.1057	.1324	.1165
.128	.0691	.1468	.1292	.1219	.1073	.1344	.1183
.130	.0702	.1490	.1311	.1237	.1089	.1364	.1201
.132	.0712	.1512	.1330	.1255	.1105	.1384	.1219
.134	.0723	.1534	.1350	.1273	.1121	.1404	.1236
.136	.0733	.1556	.1369	.1292	.1137	.1424	.1253
.138	.0743	.1578	.1389	.1310	.1153	.1444	.1271
.140	.0754	.1600	.1408	.1328	.1169	.1464	.1288
.142	.0764	.1622	.1427	.1346	.1185	.1484	.1306
.144	.0774	.1644	.1447	.1364	.1201	.1504	.1324
.146	.0785	.1666	.1466	.1383	.1217	.1525	.1342
.148	.0795	.1688	.1486	.1401	.1233	.1545	.1360
.150	.0805	.1710	.1505	.1419	.1249	.1565	.1377
.152	.0816	.1732	.1524	.1437	.1265	.1585	.1395
.154	.0826	.1754	.1544	.1455	.1281	.1605	.1413
.156	.0837	.1776	.1563	.1474	.1297	.1625	.1430
.158	.0847	.1798	.1583	.1492	.1313	.1645	.1448
.160	.0857	.1820	.1602	.1510	.1329	.1665	.1465
.162	.0868	.1842	.1621	.1528	.1345	.1685	.1483
.164	.0878	.1864	.1640	.1546	.1361	.1705	.1501
.166	.0888	.1886	.1660	.1565	.1377	.1726	.1519
.168	.0899	.1908	.1679	.1583	.1393	.1746	.1537
.170	.0909	.1930	.1698	.1601	.1409	.1766	.1554
.172	.0920	.1952	.1717	.1619	.1425	.1786	.1572
.174	.0930	.1974	.1736	.1637	.1441	.1806	.1590
.176	.0940	.1996	.1756	.1656	.1457	.1826	.1607
.178	.0951	.2018	.1775	.1674	.1473	.1846	.1625
.180	.0961	.2039	.1794	.1692	.1489	.1866	.1642
.182	.0971	.2061	.1813	.1710	.1505	.1886	.1660
.184	.0982	.2082	.1832	.1728	.1521	.1906	.1678
.186	.0992	.2104	.1851	.1747	.1537	.1926	.1695
.188	.1003	.2126	.1870	.1765	.1553	.1946	.1712
.190	.1013	.2147	.1889	.1783	.1569	.1965	.1729
.192	.1023	.2168	.1908	.1801	.1585	.1985	.1747
.194	.1034	.2190	.1927	.1819	.1601	.2005	.1764
.196	.1044	.2212	.1946	.1838	.1617	.2025	.1782
.198	.1054	.2233	.1965	.1856	.1633	.2045	.1800
.200	.1065	.2255	.1984	.1874	.1649	.2065	.1817
.202	.1075	.2276	.2003	.1892	.1665	.2085	.1835
.204	.1085	.2298	.2022	.1910	.1681	.2105	.1853
.206	.1096	.2320	.2041	.1929	.1697	.2125	.1869
.208	.1106	.2341	.2060	.1947	.1713	.2144	.1887



*Kröber's table.*—Concluded.

44.22

(Expressed in grams.)

FURFURAL PHLOROGLUCIDE	FURFURAL	ARABINOSE	ARABAN	XYLOSE	XYLAN	PENTOSE	PENTOSAN
0.210	0.1116	0.2363	0.2079	0.1965	0.1729	0.2164	0.1904
.212	.1127	.2384	.2098	.1984	.1745	.2184	.1922
.214	.1137	.2406	.2117	.2002	.1761	.2204	.1940
.216	.1147	.2428	.2136	.2020	.1778	.2224	.1957
.218	.1158	.2449	.2155	.2038	.1794	.2244	.1974
.220	.1168	.2471	.2174	.2057	.1810	.2264	.1992
.222	.1178	.2492	.2193	.2075	.1826	.2284	.2010
.224	.1189	.2514	.2212	.2093	.1842	.2304	.2028
.226	.1199	.2536	.2232	.2111	.1858	.2324	.2046
.228	.1209	.2557	.2251	.2130	.1874	.2344	.2063
.230	.1220	.2579	.2270	.2148	.1890	.2364	.2081
.232	.1230	.2600	.2289	.2166	.1906	.2383	.2097
.234	.1240	.2622	.2308	.2184	.1922	.2403	.2115
.236	.1251	.2644	.2327	.2202	.1938	.2423	.2132
.238	.1261	.2665	.2346	.2220	.1954	.2443	.2150
.240	.1271	.2687	.2365	.2239	.1970	.2463	.2168
.242	.1281	.2708	.2384	.2257	.1986	.2483	.2185
.244	.1292	.2730	.2403	.2275	.2002	.2503	.2203
.246	.1302	.2752	.2422	.2293	.2018	.2523	.2220
.248	.1312	.2773	.2441	.2311	.2034	.2543	.2238
.250	.1323	.2795	.2460	.2330	.2050	.2563	.2256
.252	.1333	.2816	.2479	.2348	.2066	.2582	.2272
.254	.1343	.2838	.2498	.2366	.2082	.2602	.2290
.256	.1354	.2860	.2517	.2384	.2098	.2622	.2307
.258	.1364	.2881	.2536	.2402	.2114	.2642	.2325
.260	.1374	.2903	.2555	.2420	.2130	.2662	.2342
.262	.1385	.2924	.2574	.2438	.2146	.2681	.2359
.264	.1395	.2946	.2593	.2456	.2162	.2701	.2377
.266	.1405	.2968	.2612	.2474	.2178	.2721	.2394
.268	.1416	.2989	.2631	.2492	.2194	.2741	.2412
.270	.1426	.3011	.2650	.2511	.2210	.2761	.2429
.272	.1436	.3032	.2669	.2529	.2226	.2781	.2447
.274	.1447	.3054	.2688	.2547	.2242	.2801	.2465
.276	.1457	.3076	.2707	.2565	.2258	.2821	.2482
.278	.1467	.3097	.2726	.2583	.2274	.2840	.2499
.280	.1478	.3119	.2745	.2602	.2290	.2861	.2517
.282	.1488	.3140	.2764	.2620	.2306	.2880	.2534
.284	.1498	.3162	.2783	.2638	.2322	.2900	.2552
.286	.1509	.3184	.2802	.2656	.2338	.2920	.2570
.288	.1519	.3205	.2821	.2674	.2354	.2940	.2587
.290	.1529	.3227	.2840	.2693	.2370	.2960	.2605
.292	.1540	.3248	.2859	.2711	.2386	.2980	.2622
.294	.1550	.3270	.2878	.2729	.2402	.3000	.2640
.296	.1560	.3292	.2897	.2747	.2418	.3020	.2658
.298	.1571	.3313	.2916	.2765	.2434	.3040	.2675
.300	.1581	.3335	.2935	.2784	.2450	.3060	.2693

44.23 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.<sup>1</sup>

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
1.0000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
.9999	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07
.98	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13
.97	.20	.20	.20	.20	.20	.20	.20	.20	.20	.20	.20	.20
.96	.27	.26	.26	.26	.26	.26	.26	.26	.26	.26	.26	.26
.95	.33	.33	.33	.33	.33	.33	.33	.33	.33	.33	.33	.33
.94	.40	.40	.40	.40	.40	.40	.40	.40	.40	.40	.40	.40
.93	.47	.46	.46	.46	.46	.46	.46	.46	.46	.46	.46	.46
.92	.53	.53	.53	.53	.53	.53	.53	.53	.53	.53	.53	.53
.91	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60
.90	.67	.66	.66	.66	.66	.66	.66	.66	.66	.66	.66	.66
.89	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73
.88	.80	.80	.80	.80	.80	.80	.79	.79	.79	.79	.79	.79
.87	.87	.87	.87	.87	.87	.87	.86	.86	.86	.86	.86	.86
.86	.93	.93	.93	.93	.93	.93	.93	.93	.93	.93	.93	.93
.85	1.00	1.00	1.00	1.00	1.00	1.00	.99	.99	.99	.99	.99	.99
.84	.07	.07	.07	.07	.07	.07	1.06	1.06	1.06	1.06	1.06	1.06
.83	.14	.14	.14	.13	.13	.13	.13	.13	.13	.13	.13	.13
.82	.20	.20	.20	.20	.20	.20	.19	.19	.19	.19	.19	.19
.81	.27	.27	.27	.27	.27	.27	.26	.26	.26	.26	.26	.26
.80	.34	.34	.34	.34	.34	.33	.33	.32	.32	.32	.32	.32
.79	.41	.41	.41	.40	.40	.40	.39	.39	.39	.39	.39	.39
.78	.48	.48	.48	.47	.47	.47	.46	.46	.46	.46	.46	.46
.77	.54	.54	.54	.54	.54	.53	.53	.53	.53	.53	.53	.53
.76	.61	.61	.61	.60	.60	.60	.59	.59	.59	.59	.59	.59
.75	.68	.68	.68	.67	.67	.67	.66	.66	.66	.66	.66	.66
.74	.75	.75	.75	.74	.74	.73	.73	.73	.73	.73	.73	.73
.73	.82	.81	.81	.81	.81	.80	.80	.80	.80	.80	.80	.80
.72	.88	.88	.88	.87	.87	.86	.86	.86	.86	.86	.86	.86
.71	.95	.95	.95	.94	.94	.94	.93	.93	.93	.92	.92	.92
.70	2.02	2.02	2.02	2.01	2.01	2.01	2.00	2.00	2.00	.99	.99	.99
.69	.09	.09	.09	.08	.08	.08	.07	.07	.06	2.05	2.05	2.05
.68	.16	.15	.15	.14	.14	.14	.14	.14	.13	.12	.12	.12
.67	.23	.22	.22	.21	.21	.21	.20	.20	.20	.19	.19	.19
.66	.30	.29	.29	.28	.28	.28	.27	.27	.27	.26	.26	.26
.65	.37	.36	.36	.35	.35	.35	.34	.34	.33	.32	.32	.32
.64	.43	.43	.43	.42	.42	.42	.41	.41	.40	.39	.39	.39
.63	.50	.50	.50	.49	.49	.49	.48	.48	.47	.46	.46	.46
.62	.57	.57	.57	.56	.56	.56	.55	.54	.54	.53	.53	.53
.61	.64	.64	.64	.63	.63	.63	.62	.61	.60	.59	.59	.59
.60	.71	.70	.70	.70	.70	.70	.69	.68	.67	.67	.66	.66
.59	.78	.77	.77	.77	.77	.77	.76	.75	.74	.74	.73	.73
.58	.85	.84	.84	.83	.83	.83	.82	.81	.81	.81	.80	.80
.57	.92	.91	.91	.90	.90	.90	.89	.88	.87	.87	.86	.86
.56	.99	.98	.98	.97	.97	.97	.96	.95	.94	.94	.93	.93
.55	3.05	3.05	3.05	3.04	3.04	3.04	3.03	3.02	3.01	3.01	3.00	3.00
.54	.13	.12	.12	.11	.11	.11	.10	.09	.08	.08	.07	.07
.53	.20	.19	.19	.18	.18	.18	.17	.16	.15	.15	.14	.14
.52	.27	.26	.26	.25	.25	.25	.24	.23	.22	.22	.21	.21
.51	.34	.33	.33	.32	.32	.32	.31	.30	.29	.28	.27	.27
.50	.41	.40	.40	.39	.39	.39	.38	.37	.36	.35	.34	.34
.49	.49	.47	.47	.46	.46	.46	.45	.44	.43	.42	.41	.41
.48	.56	.54	.54	.53	.53	.53	.52	.51	.50	.49	.48	.48
.47	.63	.61	.61	.60	.60	.60	.59	.58	.57	.56	.55	.55
.46	.70	.68	.68	.67	.67	.67	.66	.65	.64	.63	.62	.62
.45	.77	.76	.75	.74	.74	.74	.73	.72	.70	.69	.68	.68
.44	.84	.83	.82	.81	.81	.81	.79	.78	.77	.76	.75	.75
.43	.91	.90	.89	.88	.88	.88	.86	.85	.84	.83	.82	.82
.42	.99	.97	.96	.95	.95	.95	.93	.92	.91	.90	.89	.89
.41	4.06	4.04	4.03	4.02	4.02	4.02	4.00	.99	.98	.97	.96	.96
.40	.13	.11	.10	.10	.09	.09	.07	4.06	4.05	4.04	4.03	4.03
.39	.20	.18	.17	.17	.16	.16	.14	.13	.12	.11	.10	.10
.38	.28	.26	.25	.25	.24	.23	.21	.20	.19	.18	.17	.17
.37	.35	.33	.32	.32	.31	.30	.28	.27	.26	.25	.24	.24
.36	.42	.40	.39	.39	.38	.37	.36	.35	.33	.32	.31	.30
.35	.50	.48	.47	.46	.45	.44	.43	.42	.40	.39	.38	.37
.34	.57	.55	.54	.53	.52	.51	.50	.49	.47	.46	.45	.44
.33	.64	.62	.61	.60	.59	.58	.57	.56	.54	.53	.52	.51
.32	.71	.69	.68	.67	.66	.65	.64	.63	.61	.60	.59	.58
.31	.79	.77	.76	.75	.74	.73	.72	.70	.68	.67	.66	.65
.30	.86	.84	.83	.82	.81	.80	.79	.77	.75	.74	.73	.72

<sup>1</sup> Compiled at the National Bureau of Standards. The table is based on data published in the Bulletin of the Bureau of Standards, Vol. 9, No. 3 (Sci. Paper No. 197).

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued. 44.23

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9030	4.86	4.84	.83	.82	4.81	.80	.79	4.77	.75	.74	4.73	.72
29	.93	.91	.90	.89	.88	.87	.86	.84	.82	.81	.80	.79
28	5.01	.98	.97	.96	.95	.94	.93	.91	.89	.88	.87	.86
27	.08	5.06	5.04	5.03	5.02	5.01	5.00	.98	.96	.95	.94	.93
26	.16	.13	.12	.11	.10	.09	.07	5.05	5.03	5.02	5.01	5.00
25	.23	.21	.19	.18	.17	.16	.14	.12	.10	.09	.08	.07
24	.31	.28	.26	.25	.24	.23	.21	.20	.18	.16	.15	.14
23	.39	.36	.34	.33	.32	.31	.29	.27	.25	.23	.22	.21
22	.46	.43	.41	.40	.39	.38	.36	.34	.32	.30	.29	.28
21	.54	.51	.49	.48	.47	.46	.44	.42	.40	.38	.37	.36
20	.61	.58	.56	.55	.54	.53	.51	.49	.47	.45	.44	.43
19	.69	.66	.64	.62	.61	.60	.58	.56	.54	.52	.51	.50
18	.77	.73	.71	.70	.69	.68	.66	.64	.62	.59	.58	.57
17	.84	.81	.79	.77	.76	.75	.73	.71	.69	.66	.65	.64
16	.92	.88	.86	.85	.84	.83	.80	.78	.76	.74	.73	.72
15	.99	.96	.94	.92	.91	.90	.87	.85	.83	.81	.80	.79
14	6.07	6.03	6.01	6.00	.99	.98	.95	.93	.91	.88	.87	.86
13	.15	.11	.09	.07	6.06	6.05	6.02	6.00	.98	.95	.94	.93
12	.23	.18	.16	.15	.14	.13	.10	.08	6.05	6.02	6.01	6.00
11	.30	.26	.24	.22	.21	.20	.17	.15	.12	.10	.09	.08
10	.38	.34	.32	.30	.29	.28	.25	.23	.20	.17	.16	.15
09	.46	.41	.39	.37	.36	.35	.32	.30	.28	.25	.24	.23
08	.54	.49	.47	.45	.44	.43	.40	.38	.35	.32	.31	.30
07	.62	.57	.55	.53	.52	.51	.48	.45	.42	.39	.38	.37
06	.70	.65	.63	.60	.59	.58	.55	.53	.50	.47	.46	.45
05	.77	.73	.71	.68	.67	.66	.63	.60	.57	.54	.53	.52
04	.85	.80	.78	.75	.74	.73	.70	.68	.65	.62	.60	.59
03	.93	.88	.86	.83	.82	.81	.78	.75	.72	.69	.68	.67
02	7.01	.96	.93	.90	.89	.88	.85	.83	.80	.77	.75	.74
01	.09	7.04	7.01	.98	.97	.95	.92	.90	.87	.84	.82	.81
00	.17	.12	.09	7.06	7.05	7.03	7.00	.98	.94	.91	.90	.88
0.9899	.25	.19	.16	.13	.12	.10	.07	7.05	7.01	.98	.97	.95
98	.33	.27	.24	.21	.20	.18	.15	.13	.09	7.06	7.04	7.02
97	.41	.35	.32	.29	.28	.26	.23	.21	.17	.14	.12	.10
96	.50	.43	.40	.37	.36	.34	.31	.28	.24	.21	.19	.17
95	.58	.51	.48	.45	.44	.42	.39	.36	.32	.29	.27	.25
94	.66	.59	.56	.53	.52	.50	.47	.44	.40	.36	.34	.32
93	.74	.67	.64	.60	.59	.57	.54	.51	.47	.44	.42	.40
92	.82	.75	.72	.68	.67	.65	.62	.59	.55	.51	.49	.47
91	.90	.82	.79	.76	.75	.73	.70	.66	.62	.59	.57	.55
90	.98	.90	.87	.84	.83	.81	.78	.74	.70	.66	.64	.62
89	8.07	.98	.95	.92	.91	.89	.86	.82	.78	.74	.72	.70
88	.15	8.06	8.03	8.00	.98	.96	.93	.89	.85	.81	.79	.77
87	.23	.15	.11	.08	8.06	8.04	8.01	.97	.93	.89	.87	.85
86	.32	.23	.19	.16	.14	.12	.09	8.05	8.01	.96	.94	.92
85	.40	.31	.27	.24	.22	.20	.16	.12	.08	8.04	8.02	8.00
84	.48	.39	.35	.32	.30	.28	.24	.20	.16	.11	.09	.07
83	.57	.47	.43	.40	.38	.36	.32	.27	.23	.19	.17	.15
82	.65	.55	.51	.48	.46	.44	.40	.35	.31	.26	.24	.22
81	.73	.63	.59	.56	.54	.52	.48	.43	.39	.34	.32	.30
80	.82	.71	.67	.63	.61	.59	.55	.50	.46	.41	.39	.37
79	.90	.79	.75	.71	.69	.67	.63	.58	.54	.49	.47	.45
78	.98	.88	.84	.79	.77	.75	.71	.66	.61	.56	.54	.52
77	9.07	.96	.92	.87	.85	.83	.78	.73	.69	.64	.62	.60
76	.15	9.04	9.00	.95	.93	.91	.86	.81	.76	.71	.69	.67
75	.24	.13	.08	9.03	9.01	.99	.94	.89	.84	.79	.77	.75
74	.32	.21	.16	.11	.09	9.07	9.02	.96	.91	.86	.84	.82
73	.40	.29	.24	.19	.17	.15	.10	9.04	.99	.94	.92	.90
72	.49	.38	.33	.27	.25	.23	.18	.12	9.07	9.02	.99	.97
71	.57	.46	.41	.35	.33	.31	.26	.20	.15	.10	9.07	9.05
70	.66	.54	.49	.43	.41	.38	.33	.27	.22	.17	.14	.12
69	.74	.62	.57	.51	.49	.46	.41	.35	.30	.25	.22	.19
68	.82	.70	.65	.59	.57	.54	.49	.43	.37	.32	.29	.26
67	.91	.79	.74	.68	.65	.62	.57	.51	.45	.40	.37	.34
66	.99	.87	.82	.76	.73	.70	.65	.59	.53	.47	.44	.41
65	10.08	.95	.90	.84	.81	.78	.72	.66	.60	.54	.51	.48
64	.16	10.08	.98	.92	.89	.86	.80	.74	.68	.62	.59	.56
63	.25	.11	10.06	10.00	.97	.94	.88	.82	.76	.69	.66	.63
62	.33	.20	.14	.08	10.05	10.02	.96	.90	.84	.77	.74	.71
61	.42	.28	.22	.16	.13	.10	10.04	.98	.91	.84	.81	.78
60	.50	.36	.30	.24	.21	.18	.11	10.05	.99	.92	.89	.86

**44.23 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.**—Continued.

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9860	10.50	10.36	.30	.24	10.21	.18	.11	10.05	.99	.92	9.89	.86
59	.59	.44	.38	.32	.29	.26	.19	.13	10.06	.99	.96	.93
58	.68	.53	.47	.40	.37	.34	.27	.21	.14	10.07	10.04	10.00
57	.76	.61	.55	.48	.44	.41	.34	.28	.21	.14	.11	.07
56	.85	.69	.63	.56	.52	.49	.42	.36	.29	.22	.19	.15
55	.93	.78	.71	.64	.60	.57	.50	.44	.37	.30	.26	.23
54	11.02	.86	.79	.72	.68	.65	.58	.52	.45	.38	.34	.31
53	.11	.94	.87	.80	.76	.73	.66	.59	.52	.45	.41	.38
52	.19	11.03	.96	.88	.84	.81	.74	.67	.60	.53	.49	.45
51	.28	.11	11.04	.96	.92	.89	.82	.75	.67	.60	.56	.52
50	.37	.19	.12	11.04	11.00	.96	.89	.82	.74	.67	.63	.59
49	.46	.28	.20	.12	.08	11.04	.97	.90	.82	.75	.71	.67
48	.54	.36	.28	.20	.16	.12	11.05	.98	.90	.82	.78	.74
47	.63	.45	.36	.28	.24	.20	.13	11.05	.97	.90	.86	.82
46	.72	.53	.45	.37	.33	.29	.21	.13	11.05	.97	.93	.89
45	.81	.61	.53	.45	.41	.37	.29	.21	.13	11.05	11.01	.97
44	.89	.70	.62	.53	.49	.45	.37	.29	.21	.12	.08	11.04
43	.98	.78	.70	.61	.57	.53	.44	.36	.28	.20	.16	.12
42	12.07	.87	.78	.69	.65	.61	.52	.44	.36	.27	.23	.19
41	.16	.95	.86	.78	.73	.69	.60	.52	.44	.35	.31	.27
40	.25	12.04	.95	.86	.81	.77	.68	.60	.51	.42	.38	.34
39	.34	.12	12.03	.94	.89	.85	.76	.67	.58	.50	.46	.42
38	.43	.21	.12	12.03	.98	.93	.84	.75	.66	.57	.53	.49
37	.52	.30	.20	.11	12.06	12.01	.92	.83	.74	.65	.61	.57
36	.61	.38	.28	.19	.14	.09	12.00	.91	.82	.73	.68	.64
35	.70	.47	.37	.27	.22	.17	.07	.98	.89	.80	.76	.72
34	.79	.55	.45	.35	.30	.25	.15	12.06	.97	.88	.83	.79
33	.88	.64	.54	.44	.39	.34	.24	.14	12.05	.96	.91	.86
32	.97	.73	.63	.52	.47	.42	.32	.22	.12	12.03	.98	.93
31	13.06	.81	.71	.60	.55	.50	.40	.30	.20	.11	12.06	12.01
30	.16	.90	.79	.68	.63	.58	.48	.38	.28	.19	.14	.09
29	.25	.99	.88	.77	.71	.66	.56	.46	.36	.26	.21	.16
28	.34	13.07	.96	.85	.80	.74	.64	.54	.44	.34	.29	.24
27	.43	.16	13.05	.93	.88	.82	.72	.62	.52	.42	.37	.32
26	.52	.25	.13	13.01	.96	.90	.80	.70	.59	.49	.44	.39
25	.61	.34	.22	.10	13.04	.99	.88	.78	.67	.57	.52	.47
24	.71	.43	.31	.19	.13	13.08	.97	.86	.75	.65	.60	.55
23	.80	.51	.39	.27	.21	.16	13.05	.94	.83	.72	.67	.62
22	.89	.60	.47	.35	.29	.24	.13	13.02	.91	.80	.75	.70
21	.98	.68	.56	.44	.38	.33	.22	.10	.99	.88	.82	.77
20	14.08	.77	.64	.52	.46	.40	.29	.18	13.06	.95	.90	.85
19	.17	.86	.73	.61	.55	.49	.37	.26	.15	13.04	.98	.93
18	.26	.95	.82	.69	.63	.57	.45	.34	.22	.11	13.05	13.00
17	.36	14.04	.91	.78	.72	.66	.54	.42	.30	.19	.13	.08
16	.45	.13	14.00	.87	.80	.74	.62	.50	.38	.27	.21	.16
15	.55	.22	.08	.95	.88	.82	.70	.58	.46	.34	.28	.23
14	.64	.30	.17	14.04	.97	.91	.78	.66	.54	.42	.36	.30
13	.74	.39	.25	.12	14.05	.99	.86	.74	.62	.50	.44	.38
12	.83	.48	.34	.20	.13	14.07	.94	.82	.70	.58	.52	.46
11	.92	.57	.43	.29	.22	.16	14.03	.90	.77	.65	.59	.53
10	15.02	.66	.51	.37	.30	.24	.11	.98	.85	.73	.67	.61
09	.11	.75	.60	.46	.39	.32	.19	14.06	.93	.81	.75	.69
08	.21	.84	.79	.54	.47	.40	.27	.14	14.01	.88	.82	.76
07	.30	.93	.67	.62	.55	.48	.35	.22	.09	.96	.90	.84
06	.40	15.02	.76	.71	.64	.57	.43	.30	.17	14.04	.98	.92
05	.49	.11	.95	.79	.72	.65	.51	.38	.25	.12	14.05	.89
04	.58	.20	15.04	.88	.81	.74	.60	.46	.33	.20	.13	14.07
03	.67	.28	.12	.96	.89	.82	.68	.54	.41	.28	.21	.15
02	.77	.37	.21	15.05	.97	.90	.76	.62	.49	.36	.29	.23
01	.87	.46	.30	.14	15.06	.99	.84	.70	.56	.43	.36	.30
00	.96	.55	.39	.23	.15	15.07	.92	.78	.64	.51	.44	.38
0.9799	16.06	.64	.48	.32	.24	.16	15.01	.86	.72	.59	.52	.46
98	.15	.73	.46	.40	.32	.24	.09	.94	.80	.67	.60	.54
97	.25	.82	.55	.49	.41	.33	.17	15.02	.88	.74	.67	.61
96	.35	.91	.64	.57	.49	.41	.26	.11	.96	.82	.75	.68
95	.44	16.00	.83	.66	.58	.50	.34	.19	15.04	.90	.83	.76
94	.54	.10	.92	.75	.66	.59	.43	.27	.12	.98	.91	.84
93	.63	.19	16.01	.84	.75	.67	.51	.35	.20	15.05	.98	.91
92	.73	.28	.10	.83	.84	.76	.59	.43	.28	.13	15.06	.99
91	.83	.37	.19	16.01	.92	.84	.67	.51	.36	.21	.14	15.07
90	.92	.46	.27	.09	16.00	.92	.75	.59	.44	.29	.22	.15

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued. 44.23

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9790	16.92	16.46	16.27	16.09	16.00	15.92	15.75	15.59	15.44	15.29	15.22	15.15
89	17.02	.55	.26	.18	.09	16.01	.84	.67	.52	.37	.30	.23
88	.12	.64	.45	.27	.18	.10	.93	.76	.61	.45	.38	.31
87	.22	.73	.54	.36	.27	.18	16.01	.84	.68	.52	.45	.38
86.4	.32	.83	.63	.44	.35	.26	.09	.92	.76	.60	.53	.46
85	.42	.92	.72	.53	.44	.35	.17	16.00	.84	.68	.61	.53
84	.51	17.01	.81	.62	.53	.44	.26	.08	.92	.76	.69	.61
83	.61	.10	.90	.70	.61	.52	.34	.17	.10	.84	.77	.69
82	.71	.20	.99	.79	.70	.61	.43	.25	16.08	.92	.84	.76
81	.81	.29	17.08	.88	.78	.69	.51	.33	.16	16.00	.92	.84
80	.91	.38	.17	.97	.87	.78	.59	.41	.24	.08	16.00	.92
79	18.01	.47	.26	17.06	.96	.87	.68	.50	.33	.16	.08	16.00
78	.11	.57	.35	.14	17.04	.95	.76	.58	.41	.24	.16	.08
77	.21	.66	.44	.23	.13	17.04	.85	.66	.49	.32	.24	.16
76	.31	.75	.53	.32	.22	.12	.93	.74	.57	.40	.32	.24
75	.41	.84	.62	.40	.30	.20	17.01	.83	.65	.48	.40	.32
74	.51	.94	.72	.50	.39	.29	.10	.91	.73	.56	.48	.40
73	.61	18.03	.81	.59	.48	.38	.18	.99	.81	.64	.56	.48
72	.71	.12	.90	.68	.57	.47	.27	17.07	.89	.72	.63	.55
71	.81	.22	.99	.76	.65	.55	.35	.16	.97	.80	.71	.63
70	.91	.31	18.08	.85	.74	.63	.43	.24	17.05	.88	.79	.71
69	19.01	.40	.16	.94	.83	.72	.52	.32	.14	.96	.87	.79
68	.11	.50	.25	18.02	.91	.80	.60	.40	.22	17.04	.95	.86
67	.21	.59	.34	.11	18.00	.89	.69	.49	.30	.12	17.03	.94
66	.32	.69	.44	.20	.09	.98	.78	.57	.38	.20	.11	17.02
65	.42	.78	.53	.29	.18	18.07	.86	.65	.46	.28	.19	.10
64	.52	.88	.63	.38	.27	.16	.95	.74	.55	.36	.27	.17
63	.62	.97	.71	.47	.35	.24	18.03	.82	.62	.43	.35	.25
62	.72	19.07	.81	.56	.44	.33	.11	.90	.70	.51	.43	.33
61	.83	.16	.80	.65	.53	.42	.20	.98	.78	.59	.50	.41
60	.93	.26	.99	.74	.62	.50	.28	18.07	.87	.67	.58	.49
59	20.03	.35	19.08	.83	.71	.60	.37	.15	.95	.75	.66	.56
58	.13	.45	.18	.92	.80	.69	.46	.23	18.03	.83	.74	.64
57	.23	.54	.27	19.01	.88	.77	.54	.32	.11	.91	.82	.72
56	.33	.64	.36	.10	.97	.86	.62	.40	.19	.99	.90	.80
55	.43	.73	.45	.19	19.06	.94	.70	.48	.27	18.07	.98	.88
54	.53	.83	.55	.28	.15	19.03	.79	.57	.36	.15	18.06	.96
53	.63	.92	.64	.37	.24	.12	.88	.65	.44	.23	.13	18.04
52	.73	20.02	.73	.46	.33	.21	.96	.73	.52	.31	.21	.12
51	.83	.11	.82	.55	.42	.30	19.05	.82	.60	.39	.29	.19
50	.93	.20	.91	.64	.50	.38	.13	.90	.68	.47	.37	.27
49	21.03	.30	20.01	.73	.59	.47	.22	.98	.76	.55	.45	.35
48	.13	.39	.10	.82	.68	.56	.31	19.07	.85	.64	.53	.43
47	.23	.48	.19	.91	.77	.65	.39	.15	.93	.72	.61	.51
46	.33	.58	.28	20.00	.86	.74	.48	.24	19.01	.80	.69	.59
45	.43	.67	.37	.09	.95	.82	.56	.32	.09	.88	.77	.67
44	.52	.76	.46	.17	20.03	.90	.64	.40	.17	.96	.85	.75
43	.62	.86	.55	.26	.12	.99	.73	.49	.26	19.04	.93	.83
42	.72	.95	.64	.35	.21	20.08	.82	.57	.34	.12	19.01	.91
41	.82	21.04	.73	.44	.30	.17	.91	.66	.42	.20	.09	.98
40	.92	.14	.82	.53	.38	.25	.99	.74	.50	.28	.17	19.06
39	22.02	.23	.91	.62	.47	.34	20.07	.82	.58	.35	.24	.23
38	.12	.32	21.00	.71	.56	.43	.16	.90	.66	.43	.32	.31
37	.22	.41	.09	.79	.64	.51	.24	.98	.74	.51	.40	.29
36	.31	.50	.18	.88	.73	.59	.32	20.06	.82	.59	.48	.37
35	.41	.60	.27	.97	.82	.68	.41	.15	.90	.67	.56	.45
34	.51	.69	.36	21.05	.90	.77	.50	.24	.99	.75	.64	.53
33	.61	.78	.45	.14	.99	.85	.58	.32	20.07	.83	.72	.61
32	.71	.87	.54	.23	21.08	.94	.66	.40	.15	.91	.80	.68
31	.80	.96	.63	.32	.16	21.02	.74	.48	.23	.99	.87	.76
30	.90	22.05	.72	.41	.25	.11	.83	.56	.31	20.07	.95	.84
29	23.00	.14	.81	.50	.34	.20	.91	.64	.39	.15	20.03	.92
28	.10	.24	.90	.58	.42	.28	.99	.72	.47	.23	.11	20.00
27	.19	.33	.99	.67	.51	.36	21.07	.80	.55	.31	.19	.08
26	.29	.42	22.08	.76	.60	.45	.16	.89	.63	.39	.27	.16
25	.38	.51	.17	.84	.68	.53	.24	.97	.71	.46	.34	.23
24	.48	.60	.26	.93	.77	.62	.31	21.05	.84	.54	.42	.30
23	.58	.69	.34	22.01	.85	.70	.41	.13	.87	.62	.50	.38
22	.67	.78	.43	.10	.94	.78	.49	.21	.95	.70	.58	.46
21	.77	.87	.52	.19	22.03	.87	.58	.30	21.03	.78	.66	.54
20	.87	.96	.61	.27	.11	.96	.66	.38	.11	.86	.73	.61

**44.23 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.**

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9720	23.87	22.96	.61	.27	22.11	.96	.66	21.38	11	.86	20.73	.61
19	.96	23.06	.70	.36	.19	22.04	.74	.46	.19	.94	.81	.69
18	24.06	.15	.79	.45	.28	.12	.82	.54	.27	21.02	.89	.77
17	.15	.24	.88	.54	.36	.21	.91	.62	.35	.10	.97	.85
16	.25	.33	.96	.62	.45	.30	.99	.70	.43	.17	21.05	.92
15	.34	.42	23.05	.70	.53	.38	22.08	.79	.51	.24	.12	.99
14	.43	.51	.14	.79	.62	.46	.16	.87	.59	.33	.20	21.08
13	.53	.60	.22	.87	.70	.54	.24	.95	.67	.40	.27	.15
12	.62	.69	.31	.96	.79	.63	.32	22.03	.75	.88	.35	.22
11	.72	.78	.40	23.04	.87	.71	.40	.11	.83	.56	.43	.30
10	.81	.87	.49	.13	.96	.80	.49	.19	.91	.64	.50	.37
09	.91	.95	.57	.21	23.04	.88	.57	.27	.99	.72	.58	.45
08	25.00	24.04	.66	.30	.13	.97	.65	.35	22.07	.80	.66	.53
07	.09	.13	.74	.38	.21	23.05	.73	.43	.14	.87	.73	.60
06	.19	.22	.83	.47	.29	.13	.81	.51	.22	.95	.81	.68
05	.28	.31	.92	.56	.38	.22	.90	.59	.30	22.03	.89	.76
04	.38	.40	24.00	.64	.46	.30	.98	.67	.38	.10	.96	.83
03	.47	.49	.09	.73	.55	.38	23.06	.75	.46	.18	22.04	.91
02	.57	.58	.18	.81	.63	.46	.14	.83	.53	.25	.11	.98
01	.66	.66	.26	.89	.71	.54	.21	.90	.61	.33	.19	22.06
00	.75	.75	.35	.98	.80	.63	.30	.98	.69	.41	.27	.14
0.9699	.85	.84	.44	24.06	.88	.72	.38	23.06	.77	.48	.34	.21
98	.94	.93	.53	.15	.97	.80	.46	.14	.84	.55	.42	.28
97	26.04	25.01	.61	.23	24.05	.88	.54	.22	.92	.63	.49	.35
96	.13	.10	.69	.31	.13	.96	.62	.30	23.06	.71	.57	.43
95	.22	.19	.78	.40	.22	24.05	.70	.38	.08	.78	.64	.50
94	.31	.28	.86	.48	.30	.13	.78	.45	.15	.86	.72	.58
93	.41	.36	.95	.57	.38	.21	.86	.53	.23	.94	.80	.66
92	.50	.45	25.04	.65	.47	.29	.94	.61	.31	23.01	.87	.74
91	.59	.54	.13	.74	.55	.37	24.02	.69	.38	.08	.95	.81
90	.69	.62	.21	.82	.63	.45	.10	.77	.46	.16	23.02	.88
89	.78	.71	.29	.90	.72	.53	.18	.84	.53	.23	.10	.96
88	.87	.80	.38	.98	.80	.61	.26	.92	.61	.31	.17	23.03
87	.96	.89	.46	25.07	.88	.69	.34	24.00	.68	.38	.24	.10
86	27.05	.98	.55	.15	.97	.77	.42	.08	.76	.46	.32	.18
85	.15	26.06	.63	.23	25.05	.85	.50	.16	.84	.53	.39	.25
84	.24	.15	.72	.32	.13	.94	.58	.23	.92	.61	.47	.33
83	.33	.24	.80	.40	.21	25.02	.66	.31	.99	.68	.54	.40
82	.42	.33	.89	.48	.29	.10	.74	.39	24.06	.75	.61	.47
81	.51	.41	.97	.57	.37	.18	.81	.47	.14	.83	.69	.54
80	.60	.50	26.06	.65	.45	.26	.89	.54	.21	.90	.76	.61
79	.69	.59	.14	.73	.53	.34	.97	.62	.30	.98	.84	.69
78	.78	.67	.22	.81	.61	.42	25.05	.70	.37	24.06	.91	.77
77	.87	.76	.31	.89	.69	.50	.13	.78	.45	.14	.99	.84
76	.96	.84	.39	.97	.77	.58	.21	.85	.52	.21	24.06	.91
75	28.05	.93	.47	26.05	.85	.66	.29	.93	.60	.29	.13	.99
74	.14	27.01	.56	.14	.94	.74	.37	25.01	.68	.36	.21	24.06
73	.23	.10	.64	.22	26.02	.82	.45	.09	.75	.43	.28	.13
72	.32	.19	.73	.30	.10	.90	.53	.16	.83	.51	.36	.20
71	.41	.27	.81	.38	.18	.98	.60	.24	.90	.58	.43	.28
70	.50	.36	.89	.46	.26	26.06	.68	.32	.98	.66	.50	.35
69	.59	.44	.97	.54	.34	.14	.76	.40	25.06	.73	.58	.42
68	.68	.52	27.05	.63	.42	.22	.84	.47	.13	.81	.65	.50
67	.77	.61	.14	.71	.50	.30	.92	.55	.20	.88	.73	.57
66	.86	.69	.22	.79	.58	.38	.99	.63	.28	.95	.80	.64
65	.95	.77	.30	.87	.66	.46	26.07	.70	.36	25.03	.87	.72
64	29.04	.86	.39	.95	.74	.54	.15	.78	.44	.11	.95	.79
63	.12	.94	.47	27.03	.82	.62	.23	.86	.51	.18	25.02	.86
62	.21	28.02	.55	.11	.90	.70	.31	.94	.59	.25	.09	.93
61	.30	.11	.64	.19	.98	.77	.38	26.02	.66	.33	.17	25.01
60	.39	.19	.72	.27	27.06	.85	.46	.09	.74	.40	.24	.08
59	.47	.28	.81	.35	.13	.93	.54	.17	.82	.48	.31	.15
58	.56	.36	.89	.43	.21	27.01	.61	.24	.89	.56	.39	.23
57	.65	.44	.97	.51	.29	.09	.69	.32	.97	.63	.46	.30
56	.74	.53	27.05	.59	.37	.17	.77	.39	26.04	.70	.53	.37
55	.82	.61	.13	.67	.45	.25	.85	.47	.11	.77	.61	.45
54	.91	.69	.21	.75	.53	.33	.93	.55	.19	.85	.68	.52
53	30.00	.78	.29	.83	.61	.41	27.00	.62	.26	.92	.75	.59
52	.09	.86	.37	.91	.69	.49	.08	.70	.34	.99	.82	.65
51	.17	.94	.45	.99	.77	.56	.16	.78	.41	26.06	.90	.74
50	.26	29.03	.53	28.07	.85	.64	.23	.85	.49	.14	.97	.81

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued. 44.23

APPARENT SPECIFIC GRAVITY	15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
	15.56											
0.9650	30.26	29.03	.53	.07	27.85	.64	.23	26.85	.49	.14	25.97	.81
49	.34	.11	.61	.15	.93	.72	.31	.92	.56	.21	26.04	.89
48	.43	.19	.69	.23	28.01	.79	.38	27.00	.64	.29	.11	.96
47	.52	.27	.73	.31	.09	.87	.46	.07	.71	.36	.19	26.03
46	.60	.35	.85	.39	.16	.95	.53	.15	.78	.43	.26	.10
45	.69	.44	.93	.47	.24	28.03	.61	.22	.85	.51	.3	.17
44	.78	.52	29.02	.55	.32	.10	.69	.30	.93	.58	.40	.24
43	.86	.60	.10	.63	.40	.18	.76	.37	27.00	.65	.47	.31
42	.95	.68	.18	.71	.47	.26	.84	.44	.07	.72	.54	.38
41	31.03	.76	.26	.79	.55	.34	.91	.52	.14	.79	.61	.45
40	.11	.85	.34	.86	.63	.41	.99	.59	.22	.86	.69	.52
39	.20	.93	.42	.93	.71	.49	28.06	.67	.29	.93	.76	.59
38	.28	30.01	.50	29.10	.78	.56	.14	.74	.37	27.01	.83	.66
37	.36	.09	.58	.09	.86	.64	.21	.81	.44	.08	.90	.73
36	.44	.17	.66	.17	.94	.72	.29	.89	.51	.15	.97	.80
35	.52	.25	.74	.25	29.02	.80	.37	.96	.58	.22	27.04	.87
34	.61	.34	.28	.33	.09	.87	.44	28.04	.66	.29	.11	.94
33	.69	.42	.90	.41	.17	.95	.52	.11	.73	.36	.18	27.01
32	.77	.50	.98	.49	.25	29.03	.60	.19	.80	.43	.25	.08
31	.85	.58	30.06	.57	.33	.11	.67	.26	.87	.50	.32	.15
30	.93	.66	.13	.64	.40	.18	.74	.33	.95	.58	.39	.22
29	32.02	.74	.21	.72	.48	.26	.82	.41	28.02	.65	.46	.29
28	.09	.82	.29	.79	.56	.33	.89	.48	.01	.72	.54	.36
27	.17	.89	.36	.87	.64	.41	.97	.56	.17	.79	.61	.43
26	.25	.97	.44	.95	.71	.48	29.04	.63	.24	.86	.68	.50
25	.33	31.05	.52	30.03	.79	.56	.12	.70	.31	.93	.75	.57
24	.41	.13	.60	.10	.87	.64	.20	.78	.38	28.00	.82	.64
23	.49	.20	.67	.17	.95	.71	.27	.85	.45	.07	.89	.71
22	.57	.28	.75	.25	30.02	.79	.35	.93	.52	.14	.96	.78
21	.65	.36	.83	.33	.10	.86	.42	29.00	.59	.21	28.03	.85
20	.72	.44	.91	.41	.17	.94	.50	.07	.67	.29	.10	.92
19	.80	.52		.25	.25	30.01	.57	.14	.74	.36	.17	.99
18	.88	.59		.32	.32	.09	.65	.22	.82	.43	.24	28.06
17	.96	.67		.40	.40	.16	.72	.29	.89	.50	.31	.13
16	33.04	.75		.47	.47	.24	.79	.36	.96	.57	.38	.20
15	.12	.82		.54	.54	.31	.86	.43	29.03	.64	.45	.27
14	.19	.90		.62	.62	.39	.94	.51	.10	.71	.52	.43
13	.27	.98		.69	.69	.46	30.01	.58	.17	.78	.59	.41
12	.35	32.05		.77	.77	.53	.08	.65	.24	.85	.66	.48
11	.43	.13		.84	.84	.61	.15	.72	.31	.92	.73	.55
10	.50	.21			.92	.68	.23	.80	.39	.99	.80	.62
09	.58	.28			.99	.75	.30	.87	.46	29.06	.87	.69
08	.66	.36			31.07	.83	.38	.49	.53	.13	.94	.76
07	.74	.43			.13	.90	.45	30.01	.60	.20	29.01	.83
06	.81	.51			.21	.98	.52	.09	.67	.27	.08	.90
05	.89	.58			.92	31.05	.59	.16	.74	.34	.15	.97
04	.97	.66			.36	.13	.66	.23	.81	.41	.22	29.04
03	34.05	.73			.43	.20	.73	.30	.88	.48	.29	.11
02	.12	.81			.51	.28	.80	.37	.95	.55	.36	.18
01	.20	.88			.58	.35	.88	.44	30.02	.62	.43	.25
00	.27	.96			.65	.42	.95	.51	.09	.69	.50	.31
0.9599	.35	33.03			.73			.58	.16	.76	.57	.38
98	.42	.10			.80			.65	.23	.83	.63	.45
97	.50	.18			.87			.72	.30	.90	.70	.51
96	.57	.25			.95			.79	.37	.97	.77	.58
95	.65	.32			32.02			.87	.44	30.04	.84	.65
94	.72	.40			.09			.94	.51	.11	.91	.72
93	.80	.47			.16			31.01	.58	.18	.98	.79
92	.87	.54			.23			.08	.65	.25	30.05	.86
91	.95	.62			.30			.15	.72	.32	.12	.93
90	35.02	.69			.37			.22	.79	.38	.18	.99
89	.09	.76			.44			.28			.25	30.06
88	.17	.84			.51			.35			.32	.13
87	.24	.91			.58			.42			.39	.20
86	.31	.98			.65			.49			.46	.27
85	.38	34.05			.73			.56			.52	.33
84	.46	.12			.80			.63			.59	.40
83	.53	.20			.87			.70			.66	.47
82	.60	.27			.94			.77			.73	.54
81	.67	.34			33.01			.84			.80	.61
80	.75	.41			.08			.91			.86	.67

**44.23 Percentages by volume at 60° F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.**

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.9580	35.75	34.41	33.08	31.91	30.86	0.9510	40.46	39.10	37.71	36.47	35.34
79	.82	.48	.15	.98	.93	09	.52	.16	.78	.53	.40
78	.89	.56	.22	32.05	31.00	08	.58	.23	.84	.59	.46
77	.96	.63	.29	.11	.07	07	.65	.29	.90	.65	.52
76	36.04	.70	.36	.18	.13	06	.71	.35	.96	.72	.58
75	.11	.77	.43	.25	.20	05	.77	.41	38.02	.78	.64
74	.18	.84	.50	.32	.26	04	.84	.48	.09	.84	.71
73	.25	.91	.57	.38	.33	03	.90	.54	.15	.90	.77
72	.32	.98	.64	.45	.39	02	.96	.60	.21	.96	.83
71	.39	35.05	.71	.52	.46	01	41.02	.67	.27	37.02	.89
70	.46	.12	.78	.58	.53	00	.09	.73	.33	.09	.95
69	.53	.19	.85	.65	.59	0.9499	.15	.79	.40	.15	36.01
68	.60	.26	.92	.72	.66	98	.21	.85	.46	.21	.07
67	.67	.33	.99	.79	.72	97	.27	.91	.52	.27	.13
66	.74	.40	34.05	.85	.79	96	.33	.98	.58	.33	.19
65	.81	.47	.12	.92	.86	95	.40	40.04	.64	.39	.25
64	.88	.54	.19	.99	.92	94	.46	.10	.70	.45	.31
63	.95	.61	.26	33.05	.99	93	.52	.16	.77	.51	.37
62	37.02	.68	.32	.12	32.05	92	.58	.22	.83	.57	.43
61	.09	.75	.39	.19	.12	91	.64	.29	.89	.63	.49
60	.16	.82	.46	.25	.18	90	.70	.35	.95	.70	.55
59	.22	.88	.53	.32	.25	89	.77	.41	39.01	.76	.61
58	.29	.95	.59	.39	.31	88	.83	.47	.07	.82	.67
57	.36	36.02	.66	.45	.37	87	.89	.53	.13	.88	.73
56	.43	.09	.73	.52	.44	86	.95	.59	.20	.94	.79
55	.50	.15	.80	.59	.50	85	42.01	.65	.26	38.00	.85
54	.56	.22	.86	.65	.57	84	.07	.71	.32	.06	.91
53	.63	.29	.93	.72	.63	83	.13	.78	.38	.12	.97
52	.70	.36	35.00	.79	.70	82	.19	.84	.44	.18	37.03
51	.77	.42	.07	.85	.76	81	.25	.90	.50	.24	.09
50	.84	.49	.13	.92	.83	80	.31	.96	.56	.30	.15
49	.90	.56	.20	.99	.89	79	.37	41.02	.62	.36	.21
48	.97	.63	.26	34.05	.95	78	.43	.08	.68	.42	.26
47	38.04	.69	.33	.12	33.02	77	.49	.14	.74	.48	.32
46	.11	.76	.39	.18	.08	76	.55	.20	.80	.54	.38
45	.17	.83	.46	.25	.15	75	.61	.26	.87	.60	.44
44	.24	.89	.53	.31	.21	74	.67	.32	.93	.66	.50
43	.31	.96	.59	.38	.27	73	.73	.38	.99	.72	.56
42	.37	37.03	.66	.44	.34	72	.80	.44	40.05	.78	.62
41	.44	.09	.72	.51	.40	71	.86	.50	.11	.84	.68
40	.51	.16	.79	.57	.46	70	.92	.56	.17	.90	.74
39	.57	.23	.86	.64	.53	69	.98	.62	.22	.96	.79
38	.64	.29	.92	.70	.59	68	43.04	.68	.28	39.02	.85
37	.71	.36	.99	.77	.66	67	.09	.74	.34	.08	.91
36	.77	.42	36.05	.83	.72	66	.15	.80	.40	.13	.97
35	.84	.49	.12	.90	.78	65	.21	.86	.46	.19	38.03
34	.91	.56	.18	.96	.85	64	.27	.92	.52	.25	.09
33	.97	.62	.25	35.03	.91	63	.33	.98	.58	.31	.15
32	39.04	.69	.31	.09	.97	62	.39	42.04	.64	.37	.20
31	.10	.75	.38	.15	34.04	61	.45	.09	.70	.43	.26
30	.17	.82	.44	.22	.10	60	.51	.15	.76	.49	.32
29	.23	.88	.51	.28	.16	59	.57	.21	.82	.54	.38
28	.30	.95	.57	.34	.22	58	.63	.27	.88	.60	.44
27	.36	38.01	.64	.41	.29	57	.69	.33	.93	.66	.49
26	.43	.07	.70	.47	.35	56	.75	.39	.99	.72	.55
25	.49	.14	.77	.53	.41	55	.80	.45	41.05	.78	.61
24	.56	.20	.83	.59	.47	54	.86	.51	.11	.84	.67
23	.62	.27	.90	.66	.53	53	.92	.57	.17	.89	.73
22	.69	.33	.96	.72	.60	52	.98	.63	.23	.95	.78
21	.75	.39	37.02	.78	.66	51	44.04	.69	.28	40.01	.84
20	.82	.46	.09	.85	.72	50	.10	.74	.34	.07	.90
19	.88	.52	.15	.91	.78	49	.16	.80	.40	.13	.96
18	.95	.59	.21	.97	.84	48	.21	.86	.46	.18	39.02
17	40.01	.65	.28	36.04	.91	47	.27	.92	.51	.24	.07
16	.08	.72	.34	.10	.97	46	.33	.98	.57	.30	.13
15	.14	.78	.40	.16	35.04	45	.39	43.04	.63	.35	.19
14	.20	.84	.46	.22	.10	44	.45	.09	.69	.41	.24
13	.27	.91	.52	.28	.16	43	.50	.15	.75	.47	.30
12	.33	.97	.59	.35	.22	42	.56	.21	.80	.53	.36
11	.39	39.04	.65	.41	.28	41	.62	.27	.86	.58	.41
10	.46	.10	.71	.47	.34	40	.68	.33	.92	.64	.47



Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued. 44.23

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.9440	44.68	43.33	41.92	40.64	39.47	0.9370	48.53	47.20	45.81	44.52	43.33
39	.73	.39	.98	.70	.53	69	.58	.26	.86	.58	.38
38	.79	.44	42.03	.75	.59	68	.63	.31	.91	.63	.43
37	.85	.50	.09	.81	.64	67	.69	.36	.97	.68	.49
36	.91	.56	.15	.87	.70	66	.74	.42	46.02	.74	.54
35	.97	.62	.21	.93	.76	65	.79	.47	.07	.79	.59
34	45.02	.67	.26	.98	.81	64	.85	.52	.13	.84	.65
33	.08	.73	.32	41.04	.87	63	.90	.58	.18	.90	.70
32	.14	.78	.38	.10	.93	62	.95	.63	.23	.95	.75
31	.19	.85	.43	.15	.98	61	40.01	.68	.29	45.01	.81
30	.25	.90	.49	.21	40.04	60	.06	.73	.34	.06	.86
29	.31	.96	.55	.27	.09	59	.11	.79	.39	.11	.91
28	.36	44.02	.61	.32	.15	58	.16	.84	.45	.16	.97
27	.42	.07	.66	.38	.21	57	.21	.89	.50	.22	44.02
26	.47	.13	.72	.44	.26	56	.26	.94	.55	.27	.07
25	.53	.18	.78	.49	.32	55	.32	48.00	.61	.32	.13
24	.59	.24	.83	.55	.37	54	.37	.05	.66	.37	.18
23	.64	.30	.89	.60	.43	53	.42	.10	.71	.43	.23
22	.70	.35	.95	.66	.48	52	.47	.15	.77	.48	.28
21	.76	.41	43.01	.72	.54	51	.52	.21	.82	.53	.34
20	.81	.46	.06	.77	.59	50	.58	.26	.87	.58	.39
19	.87	.52	.12	.83	.65	49	.63	.31	.93	.64	.44
18	.93	.58	.17	.89	.71	48	.68	.36	.98	.69	.49
17	.98	.63	.23	.94	.76	47	.73	.41	47.03	.74	.54
16	46.04	.69	.29	42.00	.82	46	.78	.47	.08	.79	.60
15	.09	.74	.34	.06	.87	45	.83	.52	.14	.85	.65
14	.15	.80	.40	.11	.93	44	.89	.57	.19	.90	.70
13	.20	.86	.46	.17	.98	43	.94	.62	.24	.95	.75
12	.26	.91	.51	.22	41.04	42	.99	.68	.29	46.01	.81
11	.31	.97	.57	.28	.09	41	50.04	.73	.34	.06	.86
10	.37	45.03	.62	.33	.15	40	.00	.78	.40	.11	.91
09	.43	.08	.68	.39	.20	39	.14	.83	.45	.16	.96
08	.48	.14	.74	.44	.26	38	.19	.88	.50	.21	45.02
07	.54	.19	.79	.50	.31	37	.24	.94	.55	.27	.07
06	.59	.25	.85	.56	.37	36	.30	.99	.60	.32	.12
05	.65	.30	.90	.61	.42	35	.35	49.04	.66	.37	.17
04	.70	.36	.96	.67	.48	34	.40	.00	.71	.42	.22
03	.76	.42	44.02	.72	.53	33	.45	.14	.76	.47	.27
02	.81	.47	.07	.78	.59	32	.50	.19	.81	.53	.33
01	.87	.53	.13	.83	.64	31	.55	.25	.86	.58	.38
00	.92	.58	.18	.89	.70	30	.60	.30	.92	.63	.43
0.9399	.98	.64	.23	.94	.75	29	.65	.35	.97	.68	.48
98	47.03	.69	.29	43.00	.81	28	.70	.40	48.02	.73	.53
97	.09	.74	.34	.05	.86	27	.75	.45	.07	.79	.59
96	.14	.80	.40	.11	.92	26	.81	.50	.12	.84	.64
95	.19	.85	.45	.16	.97	25	.86	.55	.17	.89	.69
94	.25	.91	.51	.22	42.03	24	.91	.60	.22	.94	.74
93	.30	.96	.56	.27	.08	23	.96	.65	.28	.99	.79
92	.35	46.01	.62	.33	.14	22	51.01	.70	.33	47.05	.84
91	.41	.07	.67	.38	.19	21	.06	.75	.38	.10	.90
90	.46	.12	.73	.44	.24	20	.11	.80	.43	.15	.95
89	.52	.18	.78	.49	.30	19	.16	.85	.48	.20	46.00
88	.57	.23	.84	.55	.35	18	.21	.90	.53	.25	.05
87	.62	.29	.89	.60	.41	17	.26	.95	.58	.30	.10
86	.68	.34	.95	.66	.46	16	.31	50.00	.63	.35	.15
85	.73	.39	45.00	.71	.52	15	.36	.05	.68	.40	.20
84	.78	.45	.05	.77	.57	14	.41	.10	.73	.45	.26
83	.84	.50	.11	.82	.63	13	.46	.16	.79	.50	.31
82	.89	.56	.16	.87	.68	12	.51	.21	.84	.55	.36
81	.95	.61	.22	.93	.73	11	.56	.26	.89	.60	.41
80	48.00	.67	.27	.98	.79	10	.61	.31	.94	.65	.46
79	.05	.72	.32	44.04	.84	09	.66	.36	.99	.71	.51
78	.11	.77	.38	.09	.90	08	.71	.41	49.04	.76	.56
77	.16	.83	.43	.15	.95	07	.76	.46	.00	.81	.61
76	.21	.88	.48	.20	43.01	06	.81	.51	.14	.86	.66
75	.26	.94	.54	.25	.06	05	.86	.56	.10	.91	.71
74	.32	.99	.59	.31	.11	04	.91	.61	.24	.96	.77
73	.37	47.04	.65	.36	.17	03	.96	.66	.29	49.01	.82
72	.42	.10	.70	.41	.22	02	52.01	.71	.34	.06	.87
71	.48	.15	.75	.47	.27	01	.06	.76	.39	.11	.92
70	.53	.20	.81	.52	.33	00	.11	.81	.44	.16	.97

44.23 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.9300	52.11	50.81	49.44	48.16	46.97	0.9230	55.52	54.24	52.88	51.61	50.41
0.9299	.16	.86	.49	.21	47.02	29	.57	.29	.93	.66	.46
98	.21	.91	.54	.26	.07	28	.62	.33	.98	.71	.51
97	.26	.96	.59	.31	.12	27	.67	.38	53.03	.75	.56
96	.31	51.01	.64	.36	.17	26	.71	.43	.08	.80	.60
95	.36	.06	.69	.41	.22	25	.76	.48	.12	.85	.65
94	.41	.11	.74	.46	.27	24	.81	.53	.17	.90	.70
93	.46	.16	.79	.51	.32	23	.86	.57	.22	.95	.75
92	.51	.21	.84	.56	.37	22	.90	.62	.27	52.00	.80
91	.56	.26	.89	.61	.42	21	.95	.67	.31	.04	.85
90	.61	.31	.94	.66	.47	20	56.00	.72	.36	.09	.89
89	.66	.36	.99	.71	.52	19	.05	.77	.41	.14	.94
88	.71	.41	50.04	.76	.57	18	.09	.81	.46	.19	.99
87	.76	.46	.09	.81	.62	17	.14	.86	.50	.23	51.04
86	.81	.50	.14	.86	.67	16	.19	.91	.55	.28	.09
85	.86	.55	.19	.91	.72	15	.24	.96	.60	.33	.13
84	.91	.60	.24	.96	.77	14	.28	55.00	.65	.38	.18
83	.96	.65	.29	49.01	.82	13	.33	.05	.70	.43	.23
82	53.00	.70	.34	.06	.87	12	.38	.10	.74	.47	.27
81	.05	.75	.39	.11	.92	11	.43	.15	.79	.52	.32
80	.10	.80	.44	.16	.97	10	.47	.19	.84	.57	.37
79	.15	.85	.49	.21	48.02	09	.52	.24	.89	.62	.42
78	.20	.90	.54	.26	.07	08	.57	.29	.93	.67	.46
77	.25	.95	.59	.31	.12	07	.62	.34	.98	.71	.51
76	.30	52.00	.64	.36	.17	06	.66	.38	54.03	.76	.56
75	.35	.05	.68	.41	.22	05	.71	.43	.08	.81	.61
74	.40	.10	.73	.46	.27	04	.76	.48	.12	.86	.65
73	.45	.15	.78	.51	.32	03	.81	.53	.17	.90	.70
72	.50	.20	.83	.56	.37	02	.85	.57	.22	.95	.75
71	.54	.25	.88	.61	.42	01	.90	.62	.26	53.00	.80
70	.59	.29	.93	.66	.47	00	.95	.67	.31	.05	.84
69	.64	.34	.98	.71	.52	0.9199	57.00	.71	.36	.09	.89
68	.69	.39	51.03	.76	.57	98	.04	.76	.41	.14	.94
67	.74	.44	.08	.81	.62	97	.09	.81	.45	.19	.99
66	.79	.49	.13	.86	.67	96	.13	.86	.50	.23	52.03
65	.84	.54	.18	.91	.71	95	.18	.90	.55	.28	.08
64	.89	.59	.23	.96	.76	94	.23	.95	.59	.33	.13
63	.94	.64	.27	50.00	.81	93	.27	56.00	.64	.37	.17
62	.99	.69	.32	.05	.86	92	.32	.04	.69	.42	.22
61	54.03	.74	.37	.10	.91	91	.37	.09	.74	.47	.27
60	.08	.79	.42	.15	.96	90	.41	.14	.78	.51	.32
59	.13	.84	.47	.20	49.01	89	.46	.18	.83	.56	.36
58	.18	.89	.52	.25	.06	88	.51	.23	.88	.61	.41
57	.23	.93	.57	.30	.11	87	.55	.28	.92	.65	.46
56	.28	.98	.62	.35	.15	86	.60	.32	.97	.70	.50
55	.32	53.03	.67	.40	.20	85	.65	.37	55.02	.75	.55
54	.37	.08	.72	.44	.25	84	.69	.42	.07	.79	.60
53	.42	.13	.76	.49	.30	83	.74	.46	.11	.84	.65
52	.47	.18	.81	.54	.35	82	.79	.51	.16	.89	.69
51	.52	.22	.86	.59	.40	81	.83	.56	.21	.93	.74
50	.57	.27	.91	.64	.44	80	.88	.60	.25	.98	.79
49	.61	.32	.96	.69	.49	79	.93	.65	.30	54.03	.83
48	.66	.37	52.01	.74	.54	78	.97	.70	.35	.07	.88
47	.71	.42	.06	.79	.59	77	58.02	.74	.39	.12	.93
46	.76	.47	.11	.83	.64	76	.06	.79	.44	.17	.98
45	.81	.52	.16	.88	.69	75	.11	.84	.49	.21	53.02
44	.86	.56	.20	.93	.73	74	.16	.88	.53	.26	.07
43	.90	.61	.25	.98	.78	73	.20	.93	.58	.31	.12
42	.95	.66	.30	51.03	.83	72	.25	.97	.63	.36	.16
41	55.00	.71	.35	.08	.88	71	.29	57.02	.67	.40	.21
40	.05	.76	.40	.13	.93	70	.34	.07	.72	.45	.26
39	.10	.81	.45	.17	.98	69	.38	.11	.77	.50	.30
38	.14	.85	.50	.22	50.02	68	.43	.16	.81	.54	.35
37	.19	.90	.54	.27	.07	67	.47	.21	.86	.59	.40
36	.24	.95	.59	.32	.12	66	.52	.25	.91	.64	.44
35	.29	54.00	.64	.37	.17	65	.57	.30	.95	.68	.49
34	.33	.05	.69	.42	.22	64	.61	.35	56.00	.73	.53
33	.38	.09	.74	.46	.27	63	.66	.39	.05	.78	.58
32	.43	.14	.79	.51	.31	62	.70	.44	.09	.83	.63
31	.48	.19	.83	.56	.36	61	.75	.48	.14	.87	.67
30	.52	.24	.88	.61	.41	60	.79	.53	.18	.92	.72

*Percentages by volume at 60°F of ethyl alcohol corresponding to apparent 44.23 specific gravities at various temperatures.—Continued.*

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.9160	58.79	57.53	56.18	54.92	53.72	0.9090	61.92	60.68	59.36	58.11	56.93
50	.84	.58	.23	.96	.77	89	.96	.72	.40	.15	.97
58	.89	.62	.28	55.01	.81	88	62.01	.77	.45	.20	57.02
57	.93	.67	.32	.06	.86	87	.05	.81	.49	.24	.06
56	.98	.71	.37	.10	.91	86	.10	.86	.54	.29	.11
55	59.02	.76	.41	.15	.95	85	.14	.90	.58	.33	.15
54	.07	.81	.46	.19	54.00	84	.18	.94	.63	.38	.19
53	.11	.85	.51	.24	.05	83	.23	.99	.67	.42	.24
52	.16	.90	.55	.29	.09	82	.27	61.03	.71	.46	.28
51	.20	.94	.60	.33	.14	81	.31	.08	.76	.51	.33
50	.25	.99	.65	.38	.18	80	.36	.12	.80	.55	.37
49	.29	58.03	.69	.42	.23	79	.40	.17	.85	.60	.42
48	.34	.08	.74	.47	.28	78	.45	.21	.89	.64	.46
47	.38	.13	.78	.52	.32	77	.49	.25	.94	.69	.50
46	.43	.17	.83	.56	.37	76	.53	.30	.98	.73	.55
45	.47	.22	.88	.61	.41	75	.58	.34	60.03	.77	.59
44	.52	.26	.92	.65	.46	74	.62	.39	.07	.82	.64
43	.56	.31	.97	.70	.51	73	.66	.43	.11	.86	.68
42	.61	.35	57.01	.75	.55	72	.71	.47	.16	.91	.73
41	.65	.40	.06	.79	.60	71	.75	.52	.20	.95	.77
40	.70	.44	.10	.84	.65	70	.79	.56	.25	59.00	.81
39	.74	.49	.15	.88	.69	69	.84	.60	.29	.04	.86
38	.79	.53	.20	.93	.74	68	.88	.65	.33	.08	.90
37	.83	.58	.24	.98	.78	67	.93	.69	.38	.13	.95
36	.88	.62	.29	56.02	.83	66	.97	.74	.42	.17	.99
35	.92	.67	.33	.07	.88	65	63.01	.78	.46	.21	58.04
34	.97	.71	.38	.11	.92	64	.06	.82	.51	.26	.08
33	60.01	.76	.42	.16	.97	63	.10	.87	.55	.30	.12
32	.06	.80	.47	.21	55.01	62	.14	.91	.60	.35	.17
31	.10	.85	.51	.25	.06	61	.19	.96	.64	.39	.21
30	.15	.89	.56	.30	.11	60	.23	62.00	.68	.43	.26
29	.19	.94	.60	.34	.15	59	.27	.04	.73	.48	.30
28	.24	.98	.65	.39	.20	58	.30	.09	.77	.52	.34
27	.28	59.03	.70	.44	.24	57	.36	.13	.82	.57	.39
26	.33	.07	.74	.48	.29	56	.40	.17	.86	.61	.43
25	.37	.12	.79	.53	.33	55	.45	.22	.90	.65	.48
24	.42	.16	.83	.57	.38	54	.49	.26	.95	.70	.52
23	.46	.21	.88	.62	.42	53	.53	.30	.99	.74	.56
22	.50	.25	.92	.67	.47	52	.58	.35	61.03	.79	.61
21	.55	.30	.97	.71	.52	51	.62	.39	.08	.83	.65
20	.59	.34	58.01	.76	.56	50	.66	.43	.12	.87	.70
19	.64	.39	.06	.80	.61	49	.71	.48	.16	.92	.74
18	.68	.43	.10	.85	.65	48	.75	.52	.21	.96	.78
17	.73	.48	.15	.89	.70	47	.79	.56	.25	60.00	.83
16	.77	.52	.19	.94	.74	46	.84	.60	.29	.05	.87
15	.82	.57	.24	.99	.79	45	.88	.65	.34	.09	.92
14	.86	.61	.28	57.03	.84	44	.92	.69	.38	.14	.96
13	.91	.66	.33	.08	.88	43	.97	.73	.42	.18	59.00
12	.95	.70	.37	.12	.93	42	64.01	.78	.47	.22	.05
11	61.00	.75	.42	.17	.97	41	.05	.82	.51	.27	.09
10	.04	.79	.46	.21	56.02	40	.09	.86	.55	.31	.13
09	.08	.84	.51	.26	.06	39	.14	.91	.60	.35	.18
08	.13	.88	.55	.30	.11	38	.18	.95	.64	.40	.22
07	.17	.92	.60	.35	.15	37	.22	.99	.68	.44	.27
06	.22	.97	.64	.39	.20	36	.27	63.04	.73	.48	.31
05	.26	60.01	.69	.44	.25	35	.31	.08	.77	.53	.35
04	.30	.06	.73	.48	.29	34	.35	.12	.81	.57	.40
03	.35	.10	.78	.53	.34	33	.40	.17	.86	.62	.44
02	.39	.15	.82	.57	.38	32	.44	.21	.90	.66	.48
01	.44	.19	.87	.62	.43	31	.48	.25	.94	.70	.53
00	.48	.24	.91	.66	.47	30	.53	.30	.99	.75	.57
0.9099	.52	.28	.96	.71	.52	29	.57	.34	62.03	.79	.61
98	.57	.33	59.00	.75	.56	28	.61	.38	.07	.83	.66
97	.61	.37	.04	.80	.61	27	.66	.43	.12	.88	.70
96	.66	.41	.09	.84	.65	26	.70	.47	.16	.92	.74
95	.70	.46	.13	.89	.70	25	.74	.51	.20	.97	.79
94	.74	.50	.18	.93	.75	24	.78	.56	.24	61.01	.83
93	.79	.55	.22	.98	.79	23	.83	.60	.29	.05	.87
92	.83	.59	.27	58.02	.84	22	.87	.64	.33	.09	.92
91	.88	.64	.31	.07	.88	21	.91	.69	.37	.14	.96
90	.92	.68	.36	.11	.93	20	.96	.73	.42	.18	60.00

44.23 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.9020	64.96	63.73	62.42	61.18	60.00	0.8950	67.90	66.69	65.39	64.16	62.99
19	65.00	.77	.46	.22	.05	49	.94	.73	.44	.21	63.03
18	.04	.82	.50	.27	.09	48	.98	.77	.48	.25	.08
17	.09	.86	.55	.31	.13	47	68.02	.81	.52	.29	.12
16	.13	.90	.59	.35	.18	46	.07	.85	.56	.33	.16
15	.17	.94	.63	.40	.22	45	.11	.90	.60	.37	.20
14	.21	.99	.67	.44	.26	44	.15	.94	.64	.42	.24
13	.26	64.03	.72	.48	.30	43	.19	.98	.69	.46	.28
12	.30	.07	.76	.53	.35	42	.23	67.02	.73	.50	.33
11	.34	.11	.80	.57	.39	41	.27	.06	.77	.54	.37
10	.38	.16	.85	.61	.43	40	.31	.10	.81	.58	.41
09	.43	.20	.89	.66	.48	39	.35	.15	.85	.63	.45
08	.47	.24	.93	.70	.52	38	.39	.19	.90	.67	.49
07	.51	.28	.97	.74	.56	37	.43	.23	.94	.71	.54
06	.55	.33	63.02	.78	.61	36	.48	.27	.98	.75	.58
05	.60	.37	.06	.83	.65	35	.52	.31	66.02	.79	.62
04	.64	.41	.10	.87	.69	34	.56	.35	.06	.84	.66
03	.68	.45	.15	.91	.73	33	.60	.39	.10	.88	.70
02	.72	.50	.19	.96	.78	32	.64	.43	.15	.92	.74
01	.77	.54	.23	62.00	.82	31	.68	.47	.19	.96	.79
00	.81	.58	.27	.04	.86	30	.72	.52	.23	65.00	.83
0.8999	.85	.62	.32	.09	.91	29	.76	.56	.27	.05	.87
98	.89	.67	.36	.13	.95	28	.80	.60	.31	.09	.91
97	.94	.71	.40	.17	.99	27	.84	.64	.35	.13	.95
96	.98	.75	.44	.21	61.03	26	.89	.68	.39	.17	64.00
95	66.02	.79	.49	.26	.08	25	.93	.72	.44	.21	.04
94	.06	.84	.53	.30	.12	24	.97	.76	.48	.25	.08
93	.10	.88	.57	.34	.16	23	60.01	.80	.52	.29	.12
92	.15	.92	.62	.38	.21	22	.05	.84	.56	.34	.16
91	.19	.96	.66	.43	.25	21	.09	.89	.60	.38	.21
90	.23	65.01	.70	.47	.29	20	.13	.93	.64	.42	.25
89	.27	.05	.74	.51	.33	19	.17	.97	.68	.46	.29
88	.31	.09	.79	.55	.38	18	.21	68.01	.73	.50	.33
87	.36	.13	.83	.60	.42	17	.25	.05	.77	.54	.37
86	.40	.18	.87	.64	.46	16	.29	.09	.81	.59	.41
85	.44	.22	.91	.68	.50	15	.33	.13	.85	.63	.46
84	.48	.26	.96	.72	.55	14	.37	.17	.89	.67	.50
83	.52	.30	64.00	.77	.59	13	.41	.21	.93	.71	.54
82	.56	.35	.04	.81	.63	12	.46	.26	.98	.75	.58
81	.61	.39	.08	.85	.68	11	.50	.30	67.02	.79	.62
80	.65	.43	.13	.89	.72	10	.54	.34	.06	.83	.67
79	.69	.47	.17	.94	.76	09	.58	.38	.10	.88	.71
78	.73	.51	.21	.98	.80	08	.62	.42	.14	.92	.75
77	.77	.56	.25	63.02	.85	07	.66	.46	.18	.96	.79
76	.82	.60	.30	.06	.89	06	.70	.50	.22	66.00	.83
75	.86	.64	.34	.11	.93	05	.74	.54	.26	.04	.87
74	.90	.68	.38	.15	.97	04	.78	.58	.30	.08	.92
73	.94	.72	.42	.19	62.02	03	.82	.62	.34	.12	.96
72	.98	.77	.47	.23	.06	02	.86	.67	.39	.17	65.00
71	67.03	.81	.51	.28	.10	01	.90	.71	.43	.21	.04
70	.07	.85	.55	.32	.14	00	.94	.75	.47	.25	.08
69	.11	.89	.59	.36	.19	0.8999	.98	.79	.51	.29	.12
68	.15	.94	.64	.40	.23	98	70.02	.83	.55	.33	.17
67	.19	.98	.68	.44	.27	97	.06	.87	.59	.37	.21
66	.23	66.02	.72	.49	.31	96	.10	.91	.63	.41	.25
65	.28	.06	.76	.53	.36	95	.14	.95	.67	.45	.29
64	.32	.10	.81	.57	.40	94	.18	.99	.71	.50	.33
63	.36	.15	.85	.61	.44	93	.22	69.03	.75	.54	.37
62	.40	.19	.89	.66	.48	92	.27	.07	.80	.58	.41
61	.44	.23	.93	.70	.53	91	.31	.11	.84	.62	.45
60	.48	.27	.97	.74	.57	90	.35	.15	.88	.66	.50
59	.53	.31	65.02	.78	.61	89	.39	.19	.92	.70	.54
58	.57	.36	.06	.83	.65	88	.43	.23	.96	.74	.58
57	.61	.40	.10	.87	.69	87	.47	.27	68.00	.79	.62
56	.65	.44	.14	.91	.74	86	.51	.32	.04	.83	.66
55	.69	.48	.18	.95	.78	85	.55	.36	.08	.87	.70
54	.73	.52	.23	64.00	.82	84	.59	.40	.12	.91	.74
53	.78	.56	.27	.04	.86	83	.63	.44	.16	.95	.79
52	.82	.60	.31	.08	.91	82	.67	.48	.20	.99	.83
51	.86	.65	.35	.12	.95	81	.71	.52	.24	67.03	.87
50	.90	.69	.39	.16	.99	80	.75	.56	.28	.07	.91

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued. 44.23

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.8880	70.75	69.56	68.28	67.07	65.91	0.8810	73.50	72.34	71.09	69.89	68.74
79	.79	.60	.33	.11	.95	09	.54	.38	.13	.93	.78
78	.83	.64	.37	.15	.99	08	.58	.42	.16	.97	.82
77	.87	.68	.41	.20	66.03	07	.62	.46	.20	70.01	.86
76	.91	.72	.45	.24	.07	06	.66	.50	.24	.05	.90
75	.95	.76	.49	.28	.11	05	.70	.53	.28	.09	.94
74	.99	.80	.53	.32	.16	04	.74	.57	.32	.13	.98
73	71.03	.84	.57	.36	.20	03	.78	.61	.36	.17	69.02
72	.07	.88	.61	.40	.24	02	.81	.65	.40	.21	.06
71	.11	.92	.65	.44	.28	01	.85	.69	.44	.25	.10
70	.15	.96	.69	.48	.32	00	.89	.73	.48	.29	.14
69	.19	70.00	.73	.52	.36	0.8799	.93	.77	.52	.33	.18
68	.23	.04	.77	.56	.40	98	.97	.81	.56	.37	.22
67	.27	.08	.81	.60	.44	97	74.01	.85	.60	.41	.26
66	.31	.12	.85	.64	.48	96	.05	.88	.64	.44	.30
65	.35	.16	.89	.68	.52	95	.08	.92	.67	.48	.34
64	.38	.20	.93	.72	.56	94	.12	.96	.71	.52	.38
63	.42	.24	.98	.76	.60	93	.16	73.00	.75	.56	.42
62	.46	.28	69.02	.80	.64	92	.20	.04	.79	.60	.45
61	.50	.32	.06	.85	.69	91	.24	.08	.83	.64	.49
60	.54	.36	.10	.89	.73	90	.28	.12	.87	.68	.53
59	.58	.40	.14	.93	.77	89	.32	.16	.91	.72	.57
58	.62	.44	.18	.97	.81	88	.36	.19	.95	.76	.61
57	.66	.48	.22	68.01	.85	87	.39	.23	.99	.80	.65
56	.70	.52	.26	.05	.89	86	.43	.27	72.03	.84	.69
55	.74	.56	.30	.09	.93	85	.47	.31	.07	.88	.73
54	.78	.60	.34	.13	.97	84	.51	.35	.11	.92	.77
53	.82	.64	.38	.17	67.01	83	.55	.39	.14	.96	.81
52	.86	.68	.42	.21	.05	82	.59	.43	.18	71.00	.85
51	.90	.72	.46	.25	.09	81	.63	.47	.22	.04	.89
50	.94	.76	.50	.29	.13	80	.66	.50	.26	.07	.93
49	.98	.80	.54	.33	.17	79	.70	.54	.30	.11	.97
48	72.02	.84	.58	.37	.21	78	.74	.58	.34	.15	70.01
47	.06	.88	.62	.41	.25	77	.78	.62	.38	.19	.05
46	.10	.92	.66	.45	.29	76	.82	.66	.42	.23	.09
45	.14	.96	.70	.49	.33	75	.86	.70	.46	.27	.13
44	.18	71.00	.74	.53	.38	74	.90	.74	.49	.31	.16
43	.22	.04	.78	.57	.42	73	.93	.78	.53	.35	.20
42	.25	.08	.82	.61	.46	72	.97	.81	.57	.39	.24
41	.29	.12	.86	.65	.50	71	75.01	.85	.61	.42	.28
40	.33	.16	.90	.69	.54	70	.05	.89	.65	.46	.32
39	.37	.20	.94	.73	.58	69	.09	.93	.69	.50	.36
38	.41	.24	.98	.77	.62	68	.13	.97	.73	.54	.40
37	.45	.27	70.02	.81	.66	67	.16	74.01	.77	.58	.44
36	.49	.31	.06	.85	.70	66	.20	.05	.81	.62	.48
35	.53	.35	.10	.89	.74	65	.24	.08	.84	.66	.52
34	.57	.39	.13	.93	.78	64	.28	.12	.88	.70	.56
33	.61	.43	.17	.97	.82	63	.32	.16	.92	.74	.60
32	.65	.47	.21	69.01	.86	62	.35	.20	.96	.77	.64
31	.69	.51	.25	.05	.90	61	.39	.24	73.00	.81	.67
30	.73	.55	.29	.09	.94	60	.43	.28	.04	.85	.71
29	.76	.59	.33	.13	.98	59	.47	.32	.08	.89	.75
28	.80	.63	.37	.17	68.02	58	.51	.35	.12	.93	.79
27	.84	.67	.41	.21	.06	57	.54	.39	.15	.97	.83
26	.88	.71	.45	.25	.10	56	.58	.43	.19	72.01	.87
25	.92	.75	.49	.29	.14	55	.62	.47	.23	.05	.91
24	.96	.79	.53	.33	.18	54	.66	.51	.27	.08	.95
23	73.00	.83	.57	.37	.22	53	.70	.55	.31	.12	.99
22	.04	.87	.61	.41	.26	52	.73	.58	.35	.16	71.03
21	.08	.91	.65	.45	.30	51	.77	.62	.38	.20	.07
20	.12	.95	.69	.49	.34	50	.81	.66	.42	.24	.10
19	.16	.99	.73	.58	.38	49	.85	.70	.46	.28	.14
18	.19	72.03	.77	.61	.42	48	.89	.74	.50	.32	.18
17	.23	.07	.81	.65	.46	47	.92	.77	.54	.36	.22
16	.27	.10	.85	.69	.50	46	.96	.81	.58	.39	.26
15	.31	.14	.89	.73	.54	45	76.00	.85	.62	.43	.30
14	.35	.18	.93	.77	.58	44	.04	.89	.65	.47	.34
13	.39	.22	.97	.81	.62	43	.07	.93	.69	.51	.38
12	.43	.26	71.01	.85	.66	42	.11	.97	.73	.55	.41
11	.47	.30	.05	.89	.70	41	.15	75.00	.77	.59	.45
10	.50	.34	.09	.89	.74	40	.19	.04	.81	.63	.49

44.23 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56	20/20	25/25	30/30	35/35
0.8740	76.19	75.04	73.81	72.63	71.49	0.8670	78.78	77.66	76.45	75.29	74.17
379	.22	.08	.85	.66	.53	69	.82	.70	.49	.33	.21
38	.26	.12	.88	.70	.57	68	.85	.73	.53	.37	.24
37	.30	.16	.92	.74	.61	67	.89	.77	.56	.40	.28
36	.34	.19	.96	.78	.65	66	.93	.81	.60	.44	.32
35	.37	.23	74.00	.82	.69	65	.96	.84	.64	.48	.36
34	.41	.27	.04	.86	.72	64	79.00	.88	.68	.51	.39
33	.45	.31	.08	.90	.76	63	.04	.92	.71	.55	.43
32	.49	.35	.11	.93	.80	62	.07	.96	.75	.59	.47
31	.52	.38	.15	.97	.84	61	.11	.99	.79	.63	.51
30	.56	.42	.19	73.01	.88	60	.14	78.03	.82	.66	.55
29	.60	.46	.23	.05	.92	59	.18	.07	.86	.70	.58
28	.64	.50	.27	.09	.96	58	.22	.10	.90	.74	.62
27	.67	.54	.31	.13	72.00	57	.25	.14	.94	.78	.66
26	.71	.57	.34	.16	.03	56	.29	.17	.97	.81	.70
25	.75	.61	.38	.20	.07	55	.32	.21	77.01	.85	.73
24	.79	.65	.42	.24	.11	54	.36	.25	.05	.89	.77
23	.82	.69	.46	.28	.15	53	.40	.28	.08	.93	.81
22	.86	.73	.50	.32	.19	52	.43	.32	.12	.96	.85
21	.90	.76	.53	.35	.23	51	.47	.36	.16	76.00	.88
20	.94	.80	.57	.39	.27	50	.51	.39	.19	.04	.92
19	.97	.84	.61	.43	.30	49	.54	.43	.23	.07	.96
18	77.01	.88	.65	.47	.34	48	.58	.47	.27	.11	75.00
17	.05	.91	.69	.51	.38	47	.61	.50	.30	.15	.03
16	.09	.95	.73	.55	.42	46	.65	.54	.34	.19	.07
15	.12	.99	.76	.58	.46	45	.69	.57	.38	.22	.11
14	.16	76.03	.80	.62	.50	44	.72	.61	.41	.26	.15
13	.20	.06	.84	.66	.53	43	.76	.65	.45	.30	.18
12	.23	.10	.88	.70	.57	42	.79	.68	.49	.33	.22
11	.27	.14	.92	.74	.61	41	.83	.72	.52	.37	.26
10	.31	.18	.95	.77	.65	40	.87	.76	.56	.41	.29
09	.34	.22	.99	.81	.69	39	.90	.79	.60	.44	.33
08	.38	.25	75.03	.85	.73	38	.94	.83	.63	.48	.37
07	.42	.29	.07	.89	.77	37	.97	.86	.67	.52	.41
06	.46	.33	.10	.93	.80	36	80.01	.90	.71	.56	.44
05	.49	.37	.14	.97	.84	35	.05	.94	.74	.59	.48
04	.53	.40	.18	74.00	.88	34	.08	.97	.78	.63	.52
03	.57	.44	.22	.04	.92	33	.12	79.01	.82	.67	.56
02	.60	.48	.25	.08	.96	32	.15	.05	.85	.70	.59
01	.64	.52	.29	.12	73.00	31	.19	.08	.89	.74	.63
00	.68	.55	.33	.16	.03	30	.22	.12	.93	.78	.67
0.8699	.71	.59	.37	.19	.07	29	.26	.16	.96	.81	.71
98	.75	.63	.40	.23	.11	28	.30	.19	78.00	.85	.74
97	.79	.66	.44	.27	.15	27	.33	.23	.04	.89	.78
96	.83	.70	.48	.31	.19	26	.37	.26	.07	.93	.82
95	.86	.74	.52	.35	.22	25	.40	.30	.11	.96	.85
94	.90	.78	.55	.38	.26	24	.44	.34	.14	77.00	.89
93	.94	.81	.59	.42	.30	23	.47	.37	.18	.04	.93
92	.97	.85	.63	.46	.34	22	.51	.41	.22	.07	.97
91	78.01	.89	.67	.50	.38	21	.55	.45	.25	.11	76.00
90	.05	.92	.70	.54	.41	20	.58	.48	.29	.15	.04
89	.08	.96	.74	.57	.45	19	.62	.52	.33	.18	.08
88	.12	77.00	.78	.61	.49	18	.65	.55	.36	.22	.11
87	.16	.03	.82	.65	.53	17	.69	.59	.40	.26	.15
86	.19	.07	.85	.69	.56	16	.72	.63	.43	.29	.19
85	.23	.11	.89	.73	.60	15	.76	.66	.47	.33	.23
84	.27	.14	.93	.76	.64	14	.80	.70	.51	.36	.26
83	.30	.18	.97	.80	.68	13	.83	.73	.54	.40	.30
82	.34	.22	76.00	.84	.72	12	.87	.77	.58	.44	.34
81	.38	.26	.04	.88	.75	11	.90	.80	.62	.47	.37
80	.41	.29	.08	.92	.79	10	.94	.84	.65	.51	.41
79	.45	.33	.12	.95	.83	09	.97	.88	.69	.55	.45
78	.49	.37	.15	.99	.87	08	81.01	.91	.72	.58	.48
77	.52	.40	.19	75.03	.91	07	.05	.95	.76	.62	.52
76	.56	.44	.23	.07	.94	06	.08	.98	.80	.66	.56
75	.60	.48	.26	.10	.98	05	.12	80.02	.83	.69	.59
74	.63	.51	.30	.14	74.02	04	.15	.05	.87	.73	.63
73	.67	.55	.34	.18	.06	03	.19	.09	.91	.77	.67
72	.71	.59	.38	.22	.09	02	.22	.13	.94	.80	.70
71	.74	.62	.41	.25	.13	01	.26	.16	.98	.84	.74
70	.78	.66	.45	.29	.17	00	.29	.20	79.01	.88	.78

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent 44.23 specific gravities at various temperatures.—Continued.

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.8600	81.29	80.20	79.01	77.88	76.78	0.8530	83.73	82.66	81.50	80.38	79.30
0.8599	.33	.23	.05	.91	.82	29	.77	.69	.54	.42	.34
98	.36	.27	.09	.95	.85	28	.80	.73	.57	.45	.38
97	.40	.30	.12	.99	.89	27	.84	.76	.61	.49	.41
96	.43	.34	.16	78.02	.93	26	.87	.80	.64	.52	.45
95	.47	.38	.19	.06	.96	25	.90	.83	.68	.56	.48
94	.51	.41	.23	.09	77.00	24	.94	.87	.71	.59	.52
93	.54	.45	.27	.13	.04	23	.97	.90	.75	.63	.55
92	.58	.48	.30	.17	.07	22	84.01	.94	.78	.66	.59
91	.61	.52	.34	.20	.11	21	.04	.97	.82	.70	.62
90	.65	.55	.37	.24	.14	20	.07	83.01	.85	.73	.66
89	.68	.59	.41	.27	.18	19	.11	.04	.89	.77	.70
88	.72	.62	.44	.31	.22	18	.14	.07	.92	.81	.73
87	.75	.66	.48	.35	.25	17	.18	.11	.96	.84	.77
86	.79	.69	.52	.38	.29	16	.21	.14	.99	.88	.80
85	.82	.73	.55	.42	.33	15	.24	.18	82.03	.91	.84
84	.86	.77	.59	.45	.36	14	.28	.21	.06	.95	.87
83	.89	.80	.62	.49	.40	13	.31	.25	.10	.98	.91
82	.93	.84	.66	.53	.43	12	.34	.28	.13	81.02	.94
81	.96	.87	.70	.56	.47	11	.38	.32	.16	.05	.98
80	82.00	.91	.73	.60	.51	10	.41	.35	.20	.09	80.01
79	.03	.94	.77	.64	.54	09	.45	.39	.23	.12	.05
78	.07	.98	.80	.67	.58	08	.48	.42	.27	.16	.08
77	.10	81.01	.84	.71	.62	07	.51	.45	.30	.19	.12
76	.14	.05	.87	.74	.65	06	.55	.49	.34	.23	.15
75	.17	.08	.91	.78	.69	05	.58	.52	.37	.26	.19
74	.21	.12	.95	.82	.72	04	.61	.56	.41	.30	.23
73	.24	.16	.98	.85	.76	03	.65	.59	.44	.33	.26
72	.28	.19	80.02	.89	.80	02	.68	.62	.47	.37	.30
71	.31	.23	.05	.92	.83	01	.72	.66	.51	.40	.33
70	.35	.26	.09	.96	.87	00	.75	.69	.54	.44	.37
69	.38	.30	.12	79.00	.91	0.8499	.78	.73	.58	.47	.40
68	.42	.33	.16	.03	.94	98	.82	.76	.61	.51	.44
67	.45	.37	.20	.07	.98	97	.85	.79	.65	.54	.47
66	.49	.40	.23	.10	78.01	96	.89	.83	.68	.57	.51
65	.52	.44	.27	.14	.05	95	.92	.86	.71	.61	.54
64	.56	.47	.30	.17	.09	94	.95	.90	.75	.64	.58
63	.59	.51	.34	.21	.12	93	.99	.93	.78	.68	.61
62	.63	.54	.37	.25	.16	92	85.02	.97	.82	.71	.65
61	.66	.58	.41	.28	.19	91	.05	84.00	.85	.75	.68
60	.70	.61	.44	.32	.23	90	.09	.03	.89	.78	.72
59	.73	.65	.48	.35	.27	89	.12	.07	.92	.82	.75
58	.77	.68	.51	.39	.30	88	.15	.10	.96	.85	.79
57	.80	.72	.55	.42	.34	87	.18	.14	.99	.89	.82
56	.84	.75	.59	.46	.37	86	.22	.17	83.02	.92	.86
55	.87	.79	.62	.49	.41	85	.25	.20	.06	.96	.89
54	.91	.82	.66	.53	.45	84	.28	.24	.09	.99	.93
53	.94	.86	.69	.57	.48	83	.32	.27	.13	82.03	.96
52	.98	.89	.73	.60	.52	82	.35	.31	.16	.06	81.00
51	83.01	.93	.76	.64	.55	81	.38	.34	.20	.10	.03
50	.04	.96	.80	.67	.59	80	.42	.37	.23	.13	.07
49	.08	82.00	.83	.71	.63	79	.45	.41	.26	.17	.10
48	.11	.03	.87	.74	.66	78	.48	.44	.30	.20	.14
47	.15	.07	.90	.78	.70	77	.51	.47	.33	.24	.17
46	.18	.10	.94	.81	.73	76	.55	.51	.37	.27	.21
45	.22	.14	.98	.85	.77	75	.58	.54	.40	.30	.24
44	.25	.17	81.01	.89	.81	74	.61	.57	.43	.34	.28
43	.29	.21	.05	.92	.84	73	.65	.61	.47	.37	.31
42	.32	.24	.08	.96	.88	72	.68	.64	.50	.41	.35
41	.35	.28	.12	.99	.91	71	.71	.67	.54	.44	.38
40	.39	.31	.15	80.03	.95	70	.75	.71	.57	.48	.42
39	.42	.35	.19	.06	.99	69	.78	.74	.61	.51	.45
38	.46	.38	.22	.10	79.02	68	.81	.78	.64	.55	.49
37	.49	.42	.26	.13	.08	67	.84	.81	.67	.58	.52
36	.53	.45	.29	.17	.09	66	.88	.84	.71	.62	.56
35	.56	.49	.30	.20	.13	65	.91	.88	.74	.65	.59
34	.59	.52	.36	.24	.16	64	.94	.91	.78	.69	.63
33	.63	.55	.40	.28	.20	63	.98	.94	.81	.72	.66
32	.66	.59	.43	.31	.23	62	86.01	.98	.85	.75	.70
31	.70	.62	.47	.35	.27	61	.04	85.01	.88	.79	.73
30	.73	.66	.50	.38	.30	60	.08	.04	.91	.82	.77

**44.23 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.**

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.8460	86.08	85.04	83.91	82.82	81.77	0.8390	88.33	87.33	86.24	85.18	84.16
59	.11	.08	.95	.86	.80	89	.36	.36	.28	.22	.19
58	.14	.11	.98	.89	.84	88	.39	.39	.31	.25	.22
57	.17	.14	84.02	.93	.87	87	.43	.43	.34	.28	.26
56	.21	.18	.05	.96	.91	86	.46	.46	.37	.31	.29
55	.24	.21	.08	83.00	.94	85	.49	.49	.40	.35	.32
54	.27	.24	.12	.03	.98	84	.52	.52	.44	.38	.36
53	.30	.28	.15	.06	82.01	83	.55	.55	.47	.41	.39
52	.34	.31	.18	.10	.04	82	.58	.58	.50	.45	.42
51	.37	.34	.22	.13	.08	81	.61	.62	.53	.48	.46
50	.40	.38	.25	.17	.11	80	.65	.65	.57	.51	.49
49	.43	.41	.29	.20	.15	79	.68	.68	.60	.54	.52
48	.47	.44	.32	.23	.18	78	.71	.71	.63	.58	.55
47	.50	.48	.35	.27	.22	77	.74	.74	.66	.61	.59
46	.53	.51	.39	.30	.25	76	.77	.78	.70	.64	.62
45	.57	.54	.42	.34	.28	75	.80	.81	.73	.68	.65
44	.60	.57	.45	.37	.32	74	.83	.84	.76	.71	.69
43	.63	.61	.49	.40	.35	73	.87	.87	.79	.74	.72
42	.66	.64	.52	.44	.39	72	.90	.90	.83	.77	.75
41	.70	.67	.55	.47	.42	71	.93	.94	.86	.81	.79
40	.73	.71	.59	.51	.46	70	.96	.97	.89	.84	.82
39	.76	.74	.62	.54	.49	69	.99	88.00	.92	.87	.85
38	.79	.77	.65	.57	.52	68	89.02	.03	.95	.90	.89
37	.83	.80	.69	.61	.56	67	.05	.06	.99	.94	.92
36	.86	.84	.72	.64	.59	66	.08	.09	87.02	.97	.95
35	.89	.87	.76	.68	.63	65	.11	.13	.05	86.00	.99
34	.92	.90	.79	.71	.66	64	.14	.16	.08	.04	85.02
33	.96	.94	.82	.74	.70	63	.18	.19	.11	.07	.05
32	.99	.97	.86	.78	.73	62	.21	.22	.15	.10	.08
31	87.02	86.00	.89	.81	.76	61	.24	.25	.18	.13	.12
30	.05	.03	.92	.85	.80	60	.27	.29	.21	.16	.15
29	.09	.07	.96	.88	.83	59	.30	.32	.24	.20	.18
28	.12	.10	.99	.91	.87	58	.33	.35	.27	.23	.22
27	.15	.13	85.02	.95	.90	57	.36	.38	.31	.26	.25
26	.18	.16	.06	.98	.93	56	.39	.41	.34	.29	.28
25	.22	.20	.09	84.02	.97	55	.42	.44	.37	.33	.31
24	.25	.23	.12	.05	83.00	54	.45	.47	.40	.36	.35
23	.28	.26	.16	.08	.04	53	.48	.50	.43	.39	.38
22	.31	.30	.19	.12	.07	52	.51	.54	.46	.42	.41
21	.34	.33	.22	.15	.11	51	.54	.57	.50	.45	.44
20	.38	.36	.25	.18	.14	50	.58	.60	.53	.49	.48
19	.41	.39	.29	.22	.17	49	.61	.63	.56	.52	.51
18	.44	.43	.32	.25	.21	48	.64	.66	.59	.55	.54
17	.47	.46	.35	.28	.24	47	.67	.69	.62	.58	.58
16	.50	.49	.39	.32	.28	46	.70	.72	.66	.62	.61
15	.54	.52	.42	.35	.31	45	.73	.75	.69	.65	.64
14	.57	.56	.45	.38	.34	44	.76	.79	.72	.68	.67
13	.60	.59	.49	.42	.38	43	.79	.82	.75	.71	.71
12	.63	.62	.52	.45	.41	42	.82	.85	.78	.75	.74
11	.67	.65	.55	.48	.45	41	.85	.88	.82	.78	.77
10	.70	.68	.59	.52	.48	40	.88	.91	.85	.81	.80
09	.73	.72	.62	.55	.51	39	.91	.94	.88	.84	.84
08	.76	.75	.65	.59	.55	38	.94	.97	.91	.87	.87
07	.79	.78	.69	.62	.58	37	.98	89.00	.94	.91	.90
06	.83	.81	.72	.65	.62	36	90.01	.04	.97	.94	.93
05	.86	.85	.75	.69	.65	35	.04	.07	88.01	.97	.97
04	.89	.88	.78	.72	.68	34	.07	.10	.04	87.00	.97
03	.92	.91	.82	.75	.72	33	.10	.13	.07	.04	.03
02	.95	.94	.85	.79	.75	32	.13	.16	.10	.07	.06
01	.99	.98	.88	.82	.79	31	.16	.19	.13	.10	.10
00	88.02	87.01	.92	.85	.82	30	.19	.22	.16	.13	.13
0.8399	.05	.04	.95	.89	.85	29	.22	.25	.19	.16	.16
98	.08	.07	.98	.92	.89	28	.25	.28	.23	.19	.19
97	.11	.10	86.02	.95	.92	27	.28	.31	.26	.23	.23
96	.14	.14	.05	.99	.96	26	.31	.35	.29	.26	.26
95	.18	.17	.08	85.02	.99	25	.34	.38	.32	.29	.29
94	.21	.20	.11	.05	84.02	24	.37	.41	.35	.32	.32
93	.24	.23	.15	.09	.06	23	.40	.44	.38	.35	.35
92	.27	.27	.18	.12	.09	22	.43	.47	.41	.39	.39
91	.30	.30	.21	.15	.12	21	.46	.50	.45	.42	.42
90	.33	.33	.24	.18	.16	20	.49	.53	.48	.45	.45



Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued. 44.23

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.8320	90.49	89.53	88.48	87.45	86.45	0.8250	92.53	91.62	90.61	89.64	88.67
19	.51	.56	.51	.48	.48	49	.55	.64	.64	.67	.70
18	.54	.59	.54	.51	.52	48	.58	.67	.67	.70	.73
17	.57	.62	.57	.54	.55	47	.61	.70	.70	.73	.76
16	.60	.65	.60	.58	.58	46	.64	.73	.73	.76	.79
15	.63	.68	.64	.61	.61	45	.66	.76	.76	.79	.83
14	.66	.71	.67	.64	.65	44	.69	.79	.79	.82	.86
13	.69	.74	.70	.67	.68	43	.72	.82	.82	.85	.89
12	.72	.77	.73	.70	.71	42	.75	.85	.85	.88	.92
11	.75	.80	.76	.74	.74	41	.78	.87	.88	.91	.95
10	.78	.83	.79	.77	.77	40	.80	.90	.91	.94	.98
09	.81	.86	.82	.80	.81	39	.83	.93	.94	.97	89.01
08	.84	.89	.85	.83	.84	38	.86	.96	.97	90.00	.04
07	.87	.93	.88	.86	.87	37	.89	.99	91.00	.03	.07
06	.90	.96	.92	.89	.90	36	.92	92.02	.03	.06	.10
05	.93	.99	.95	.93	.94	35	.94	.05	.06	.09	.13
04	.96	90.02	.98	.96	.97	34	.97	.08	.00	.12	.16
03	.99	.05	89.01	.99	87.00	33	93.00	.10	.12	.15	.20
02	91.02	.08	.04	88.02	.03	32	.03	.13	.15	.18	.23
01	.05	.11	.07	.05	.06	31	.05	.16	.18	.21	.26
00	.08	.14	.10	.08	.10	30	.08	.19	.21	.24	.29
0.8299	.11	.17	.13	.12	.13	29	.11	.22	.23	.27	.32
98	.14	.20	.16	.15	.16	28	.14	.25	.26	.30	.35
97	.17	.23	.19	.18	.19	27	.16	.28	.29	.33	.38
96	.20	.26	.22	.21	.22	26	.19	.31	.32	.36	.41
95	.23	.29	.25	.24	.25	25	.22	.33	.35	.39	.44
94	.26	.32	.28	.27	.29	24	.25	.36	.38	.42	.47
93	.28	.35	.31	.30	.32	23	.27	.39	.41	.45	.50
92	.31	.38	.35	.34	.35	22	.30	.42	.44	.48	.53
91	.34	.41	.38	.37	.38	21	.33	.45	.47	.51	.56
90	.37	.44	.41	.40	.41	20	.36	.48	.50	.54	.59
89	.40	.47	.44	.43	.44	19	.38	.50	.52	.57	.62
88	.43	.50	.47	.46	.48	18	.41	.53	.55	.60	.65
87	.46	.53	.50	.49	.51	17	.44	.56	.58	.63	.68
86	.49	.56	.53	.52	.54	16	.47	.59	.61	.66	.71
85	.52	.59	.56	.55	.57	15	.49	.62	.64	.69	.74
84	.55	.62	.59	.59	.60	14	.52	.65	.67	.72	.77
83	.58	.65	.62	.62	.63	13	.55	.67	.70	.75	.80
82	.60	.67	.65	.65	.67	12	.58	.70	.73	.78	.84
81	.63	.70	.68	.68	.70	11	.60	.73	.76	.81	.87
80	.66	.73	.71	.71	.73	10	.63	.76	.79	.84	.90
79	.69	.76	.74	.74	.76	9	.66	.79	.81	.87	.93
78	.72	.79	.77	.77	.79	08	.68	.81	.84	.90	.96
77	.75	.82	.80	.81	.83	07	.71	.84	.87	.93	.99
76	.78	.85	.83	.84	.86	06	.74	.87	.90	.96	90.02
75	.81	.88	.87	.87	.89	05	.76	.90	.93	.99	.05
74	.84	.91	.90	.90	.92	04	.79	.92	.96	91.01	.08
73	.87	.94	.93	.93	.95	03	.82	.95	.99	.04	.11
72	.90	.97	.96	.96	.98	02	.84	.98	92.02	.07	.14
71	.92	91.00	.99	.99	88.02	01	.87	93.01	.05	.10	.17
70	.95	.03	90.02	89.02	.05	00	.90	.04	.07	.13	.20
69	.98	.06	.09	.06	.08	0.8199	.92	.06	.10	.16	.23
68	92.01	.09	.08	.09	.11	98	.95	.09	.13	.19	.26
67	.04	.12	.11	.12	.14	97	.98	.12	.16	.22	.29
66	.07	.15	.14	.15	.17	96	94.01	.14	.19	.25	.32
65	.10	.18	.17	.18	.20	95	.03	.17	.22	.27	.35
64	.13	.21	.20	.21	.23	94	.06	.20	.24	.30	.38
63	.15	.24	.23	.24	.26	93	.08	.23	.27	.33	.40
62	.18	.27	.26	.27	.30	92	.11	.25	.30	.36	.43
61	.21	.29	.29	.30	.33	91	.14	.28	.33	.39	.46
60	.24	.32	.32	.33	.36	90	.16	.31	.36	.42	.49
59	.27	.35	.35	.36	.39	89	.19	.34	.39	.45	.52
58	.30	.38	.38	.39	.42	88	.22	.36	.41	.48	.55
57	.33	.41	.41	.42	.45	87	.24	.39	.44	.51	.58
56	.35	.44	.44	.45	.48	86	.27	.42	.47	.53	.61
55	.38	.47	.47	.48	.51	85	.30	.44	.50	.56	.64
54	.41	.50	.50	.51	.55	84	.32	.47	.53	.59	.67
53	.44	.53	.53	.54	.58	83	.35	.50	.56	.62	.70
52	.47	.56	.56	.57	.61	82	.38	.53	.58	.65	.73
51	.50	.59	.59	.61	.64	81	.40	.55	.61	.68	.76
50	.53	.62	.61	.64	.67	80	.43	.58	.64	.71	.79

**4.23 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued.**

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.8180	94.43	93.58	92.64	91.71	90.79	0.8110	96.20	95.42	94.53	93.67	92.80
79	.46	.61	.67	.74	.82	09	.23	.44	.56	.69	.83
78	.48	.64	.70	.77	.85	08	.25	.47	.59	.72	.86
77	.51	.66	.72	.79	.88	07	.28	.49	.61	.75	.89
76	.53	.69	.75	.82	.91	06	.30	.52	.64	.77	.92
75	.56	.72	.78	.85	.94	05	.32	.54	.66	.80	.94
74	.59	.74	.81	.88	.97	04	.35	.57	.69	.83	.97
73	.61	.77	.84	.91	91 00	03	.37	.59	.72	.85	93 00
72	.64	.80	.86	.94	03	02	.40	.62	.74	.88	03
71	.66	.82	.89	.97	.06	01	.42	.64	.77	.91	.05
70	.69	.85	.92	92.00	.09	00	.45	.67	.79	.94	08
69	.72	.88	.95	.03	.12	0.8009	.47	.69	.82	.96	.11
68	.74	.90	.97	.05	.14	98	.50	.72	.85	.99	.14
67	.77	.93	93.00	.08	.17	97	.52	.74	.87	94.02	.16
66	.79	.96	.03	.11	.20	96	.54	.77	.90	.04	.19
65	.82	.98	.06	.14	.23	95	.57	.79	.92	.07	.22
64	.84	94.01	.09	.17	.26	94	.59	.82	.95	.10	.25
63	.87	.04	.11	.20	.29	93	.61	.84	.98	.12	.27
62	.90	.06	.14	.22	.32	92	.64	.87	95 00	.15	.30
61	.92	.09	.17	.25	.35	91	.66	.89	.03	.17	.33
60	.95	.12	.20	.28	.38	90	.69	.92	.05	.20	.36
59	.97	.14	.22	.31	.40	89	.71	.94	.08	.23	.38
58	95.00	.17	.25	.34	.43	88	.73	.97	.10	.25	.41
57	.03	.20	.28	.36	.46	87	.76	.99	.13	.28	.44
56	.05	.22	.30	.39	.49	86	.78	96.02	.16	.31	.46
55	.08	.25	.33	.42	.52	85	.81	.04	.18	.33	.49
54	.10	.28	.36	.45	.55	84	.83	.07	.21	.36	.52
53	.13	.30	.39	.48	.58	83	.85	.09	.23	.39	.55
52	.15	.33	.41	.51	.61	82	.88	.11	.26	.41	.57
51	.18	.36	.44	.54	.64	81	.90	.14	.28	.44	.60
50	.20	.38	.47	.56	.66	80	.93	.16	.31	.47	.63
49	.23	.41	.50	.59	.69	79	.95	.19	.33	.49	.65
48	.25	.44	.52	.62	.72	78	.97	.21	.36	.52	.68
47	.28	.46	.55	.65	.75	77	97 00	.24	.39	.54	.71
46	.30	.49	.58	.68	.78	76	.02	.26	.41	.57	.73
45	.33	.51	.60	.70	.81	75	.04	.29	.44	.60	.76
44	.36	.54	.63	.73	.84	74	.07	.31	.46	.62	.79
43	.38	.57	.66	.76	.87	73	.09	.33	.49	.65	.81
42	.41	.59	.69	.79	.90	72	.11	.36	.51	.67	.84
41	.43	.62	.71	.82	.92	71	.14	.38	.54	.70	.87
40	.46	.64	.74	.84	.95	70	.16	.41	.56	.73	.90
39	.48	.67	.77	.87	.98	69	.18	.43	.59	.75	.92
38	.51	.70	.79	.90	92.01	68	.21	.46	.61	.78	.95
37	.53	.72	.82	.93	.04	67	.23	.48	.64	.80	.98
36	.56	.75	.85	.96	.07	66	.25	.50	.66	.83	94 00
35	.58	.77	.87	.98	.10	65	.28	.53	.69	.86	.03
34	.61	.80	.90	93.01	.13	64	.30	.55	.71	.88	.06
33	.63	.83	.93	.04	.15	63	.32	.58	.74	.91	.08
32	.66	.85	.95	.07	.18	62	.35	.60	.76	.93	.11
31	.68	.88	.98	.09	.21	61	.37	.63	.79	.96	.14
30	.71	.90	94.01	.12	.24	60	.39	.65	.81	.99	.16
29	.73	.93	.03	.15	.27	59	.42	.67	.84	95 01	.19
28	.76	.95	.06	.18	.30	58	.44	.70	.86	.04	.22
27	.78	.98	.09	.20	.33	57	.46	.72	.89	.06	.24
26	.81	95.01	.11	.23	.35	56	.49	.75	.91	.09	.27
25	.83	.03	.14	.26	.38	55	.51	.77	.94	.11	.30
24	.86	.06	.17	.29	.41	54	.53	.79	.96	.14	.32
23	.88	.08	.19	.31	.44	53	.55	.82	.99	.17	.35
22	.91	.11	.22	.34	.47	52	.58	.84	96 01	.19	.38
21	.93	.14	.24	.37	.50	51	.60	.86	.04	.22	.40
20	.96	.16	.27	.40	.53	50	.62	.89	.06	.24	.43
19	.98	.19	.30	.42	.55	49	.64	.91	.09	.27	.45
18	96.01	.21	.32	.45	.58	48	.67	.94	.11	.29	.48
17	.03	.24	.35	.48	.61	47	.69	.96	.14	.32	.51
16	.06	.26	.38	.51	.64	46	.71	.98	.16	.34	.53
15	.08	.29	.40	.53	.66	45	.73	97.01	.19	.37	.56
14	.10	.32	.43	.56	.69	44	.76	.03	.21	.40	.59
13	.13	.34	.46	.59	.72	43	.78	.05	.24	.42	.61
12	.15	.37	.48	.61	.75	42	.80	.08	.26	.45	.64
11	.18	.39	.51	.64	.78	41	.82	.10	.28	.47	.66
10	.20	.42	.53	.67	.80	40	.85	.12	.31	.50	.69

Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Continued. 44.23

APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 15.56	20/20	25/25	30/30	35/35
0.8040	97.85	97.12	96.31	95.50	94.69	0.7970	99.33	98.68	97.95	97.21	96.46
39	.87	.15	.33	.52	.72	69	.35	.70	.97	.23	.48
38	.89	.17	.36	.55	.74	68	.37	.72	.99	.25	.51
37	.91	.19	.38	.57	.77	67	.39	.75	98.02	.28	.53
36	.94	.22	.41	.60	.79	66	.42	.77	.04	.30	.56
35	.96	.24	.43	.62	.82	65	.44	.79	.06	.32	.58
34	.98	.26	.46	.65	.85	64	.46	.81	.08	.35	.60
33	98.00	.29	.48	.67	.87	63	.48	.83	.10	.37	.63
32	.03	.31	.50	.70	.90	62	.50	.85	.12	.39	.65
31	.05	.33	.53	.72	.92	61	.52	.87	.15	.42	.68
30	.07	.36	.55	.75	.95	60	.54	.89	.17	.44	.70
29	.09	.38	.58	.77	.98	59	.56	.91	.19	.46	.72
28	.11	.40	.60	.80	95.00	58	.58	.93	.21	.49	.75
27	.14	.43	.62	.82	.03	57	.60	.95	.23	.51	.77
26	.16	.45	.65	.85	.05	56	.61	.97	.26	.53	.80
25	.18	.47	.67	.87	.08	55	.63	99.00	.28	.56	.82
24	.20	.49	.70	.90	.11	54	.65	.02	.30	.58	.84
23	.22	.52	.72	.92	.13	53	.67	.04	.32	.60	.87
22	.25	.54	.74	.95	.16	52	.69	.06	.34	.62	.89
21	.27	.56	.77	.97	.18	51	.71	.08	.36	.65	.92
20	.29	.59	.79	96.00	.21	50	.73	.10	.39	.67	.94
19	.31	.61	.82	.02	.23	49	.75	.12	.41	.69	.96
18	.33	.63	.84	.05	.26	48	.77	.14	.43	.71	.99
17	.35	.66	.86	.07	.28	47	.79	.16	.45	.74	97.01
16	.38	.68	.89	.10	.31	46	.81	.18	.47	.76	.04
15	.40	.70	.91	.12	.34	45	.83	.20	.49	.78	.06
14	.42	.72	.94	.15	.36	44	.85	.22	.51	.80	.08
13	.44	.75	.96	.17	.39	43	.87	.24	.54	.83	.11
12	.46	.77	.98	.20	.41	42	.89	.26	.56	.85	.13
11	.48	.79	97.01	.22	.44	41	.91	.28	.58	.87	.15
10	.50	.81	.03	.25	.46	40	.93	.30	.60	.89	.18
09	.53	.84	.05	.27	.49	39	.95	.32	.62	.92	.20
08	.55	.86	.08	.29	.52	38	.97	.34	.64	.94	.22
07	.57	.88	.10	.32	.54	37	.99	.36	.66	.96	.25
06	.59	.90	.12	.34	.57	36	100.00	.38	.68	.98	.27
05	.61	.92	.15	.37	.59	35		.40	.70	98.01	.29
04	.63	.95	.17	.39	.62	34		.42	.73	.03	.32
03	.65	.97	.19	.42	.64	33		.44	.75	.05	.34
02	.67	.99	.22	.44	.67	32		.46	.77	.07	.36
01	.70	98.01	.24	.47	.69	31		.48	.79	.09	.39
00	.72	.03	.26	.49	.72	30		.50	.81	.12	.41
0.7999	.74	.06	.29	.51	.74	29		.52	.83	.14	.43
98	.76	.08	.31	.54	.77	28		.54	.85	.16	.46
97	.78	.10	.33	.56	.79	27		.56	.87	.18	.48
96	.80	.12	.36	.59	.82	26		.58	.89	.20	.50
95	.82	.14	.38	.61	.84	25		.60	.91	.23	.52
94	.84	.17	.40	.63	.87	24		.62	.93	.25	.55
93	.86	.19	.43	.66	.89	23		.64	.96	.27	.57
92	.88	.21	.45	.68	.92	22		.66	.98	.29	.59
91	.90	.23	.47	.71	.94	21		.68	99.00	.31	.62
90	.92	.26	.50	.73	.97	20		.70	.02	.33	.64
89	.95	.28	.52	.75	.99	19		.72	.04	.36	.66
88	.97	.30	.54	.78	96.02	18		.74	.06	.38	.68
87	.99	.32	.57	.80	.04	17		.76	.08	.40	.71
86	99.01	.34	.59	.83	.07	16		.78	.10	.42	.73
85	.03	.36	.61	.85	.09	15		.80	.12	.44	.75
84	.05	.39	.63	.87	.12	14		.82	.14	.46	.77
83	.07	.41	.66	.90	.14	13		.84	.16	.48	.80
82	.09	.43	.68	.92	.16	12		.86	.18	.51	.82
81	.11	.45	.70	.95	.19	11		.88	.20	.53	.84
80	.13	.47	.72	.97	.21	10		.90	.22	.55	.86
79	.15	.49	.75	97.00	.24	09		.92	.24	.57	.89
78	.17	.51	.77	.02	.26	08		.94	.27	.59	.91
77	.19	.54	.79	.04	.29	07		.96	.29	.61	.93
76	.21	.56	.81	.07	.31	06		.98	.31	.63	.95
75	.23	.58	.84	.09	.34	05		100.00	.33	.66	.98
74	.25	.60	.86	.11	.36	04			.35	.68	98.00
73	.27	.62	.88	.14	.38	03			.37	.70	.02
72	.29	.64	.90	.16	.41	02			.39	.72	.04
71	.31	.66	.93	.18	.43	01			.41	.74	.07
70	.33	.68	.95	.21	.46	00			.43	.76	.09

**44.23 Percentages by volume at 60°F of ethyl alcohol corresponding to apparent specific gravities at various temperatures.—Concluded.**

APPARENT SPECIFIC GRAVITY	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	35/35
0.7900	99.43	98.76	98.09	0.7830	99.56
.7899	.45	.78	.11	29	.58
98	.47	.80	.13	28	.60
97	.49	.82	.15	27	.62
96	.51	.84	.18	26	.64
95	.53	.87	.20	25	.66
94	.55	.89	.22	24	.68
93	.57	.91	.24	23	.70
92	.59	.93	.26	22	.72
91	.61	.95	.29	21	.74
90	.63	.97	.31	20	.76
89	.65	.99	.33	19	.78
88	.67	99.01	.35	18	.80
87	.69	.03	.37	17	.82
86	.71	.05	.40	16	.84
85	.73	.07	.42	15	.86
84	.75	.09	.44	14	.88
83	.77	.12	.46	13	.90
82	.79	.14	.48	12	.92
81	.81	.16	.50	11	.94
80	.83	.18	.53	09	.98
79	.85	.20	.55	08	100.00
78	.86	.22	.57		
77	.88	.24	.59		
76	.90	.26	.61		
75	.92	.28	.63		
74	.94	.30	.65		
73	.96	.32	.67		
72	.98	.34	.70		
71	100.00	.36	.72		
70		.38	.74		
69		.40	.76		
68		.42	.78		
67		.44	.80		
66		.46	.82		
65		.48	.84		
64		.50	.86		
63		.52	.89		
62		.54	.91		
61		.56	.93		
60		.58	.95		
59		.60	.97		
58		.62	.99		
57		.64	99.01		
56		.66	.03		
55		.68	.05		
54		.70	.07		
53		.72	.09		
52		.74	.12		
51		.76	.14		
50		.78	.16		
49		.80	.18		
48		.72	.20		
47		.84	.22		
46		.86	.24		
45		.88	.26		
44		.90	.28		
43		.92	.30		
42		.94	.32		
41		.96	.34		
40		.98	.36		
39		100.00	.38		
38			.40		
37			.42		
36			.44		
35			.46		
34			.48		
33			.50		
32			.52		
31			.54		
30			.56		

*Alcohol table for calculating the percentages of alcohol by volume at 15.56°C 44.24  
in mixtures of ethyl alcohol and water from their Zeiss immersion  
refractometer readings and indices of refraction at 17.5–25°C.<sup>1</sup>*

SCALE READING <sup>2</sup>	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
13.2	1.33250	....	....	....	....	....	....	....	....	0.00
13.4	3257	....	....	....	....	....	....	....	....	0.18
13.6	3265	....	....	....	....	....	....	....	0.14	0.35
13.8	3273	....	....	....	....	....	....	0.10	0.31	0.53
14.0	3281	....	....	....	....	....	0.08	0.28	0.49	0.70
14.2	3288	....	....	....	....	0.04	0.24	0.45	0.67	0.88
14.4	3296	....	....	....	....	0.21	0.41	0.63	0.84	1.06
14.6	3304	....	....	....	0.16	0.38	0.59	0.80	1.02	1.24
14.8	3312	....	....	0.14	0.34	0.55	0.77	0.98	1.19	1.40
15.0	3319	0.00	0.10	0.31	0.52	0.73	0.94	1.16	1.36	1.55
15.2	3327	0.17	0.27	0.48	0.69	0.91	1.12	1.32	1.51	1.71
15.4	3335	0.34	0.44	0.65	0.85	1.07	1.29	1.47	1.66	1.86
15.6	3343	0.51	0.60	0.82	1.03	1.24	1.44	1.62	1.82	2.01
15.8	3350	0.68	0.78	0.99	1.21	1.40	1.60	1.77	1.97	2.17
16.0	3358	0.84	0.94	1.17	1.36	1.55	1.75	1.92	2.12	2.33
16.2	3366	1.02	1.12	1.32	1.51	1.70	1.90	2.08	2.27	2.48
16.4	3374	1.18	1.29	1.47	1.66	1.85	2.05	2.24	2.43	2.62
16.6	3381	1.34	1.43	1.62	1.81	2.00	2.20	2.39	2.57	2.77
16.8	3389	1.49	1.57	1.77	1.96	2.15	2.35	2.53	2.72	2.92
17.0	3397	1.63	1.72	1.92	2.11	2.30	2.50	2.69	2.87	3.06
17.2	3405	1.77	1.87	2.06	2.26	2.45	2.65	2.82	3.02	3.21
17.4	3412	1.92	2.01	2.21	2.41	2.59	2.79	2.97	3.17	3.36
17.6	3420	2.07	2.16	2.36	2.56	2.74	2.94	3.12	3.32	3.51
17.8	3428	2.21	2.31	2.51	2.70	2.89	3.09	3.27	3.46	3.66
18.0	3435	2.36	2.45	2.66	2.85	3.04	3.23	3.42	3.61	3.81
18.2	3443	2.50	2.60	2.81	3.00	3.19	3.37	3.57	3.76	3.96
18.4	3451	2.65	2.75	2.96	3.15	3.34	3.52	3.71	3.91	4.11
18.6	3459	2.80	2.90	3.10	3.30	3.48	3.66	3.86	4.06	4.26
18.8	3466	2.95	3.05	3.25	3.45	3.63	3.81	4.01	4.21	4.41
19.0	3474	3.10	3.19	3.40	3.59	3.77	3.96	4.16	4.36	4.56
19.2	3482	3.25	3.34	3.55	3.73	3.92	4.11	4.31	4.51	4.70
19.4	3489	3.39	3.48	3.70	3.88	4.07	4.26	4.46	4.65	4.85
19.6	3497	3.53	3.63	3.84	4.03	4.22	4.41	4.61	4.80	5.00
19.8	3505	3.68	3.78	3.98	4.17	4.37	4.56	4.75	4.95	5.15
20.0	3513	3.83	3.93	4.13	4.32	4.52	4.72	4.90	5.10	5.29
20.2	3520	3.97	4.07	4.27	4.47	4.66	4.87	5.05	5.24	5.44
20.4	3528	4.12	4.22	4.42	4.61	4.82	5.01	5.20	5.38	5.58
20.6	3536	4.26	4.36	4.56	4.75	4.96	5.15	5.34	5.52	5.72
20.8	3543	4.41	4.51	4.70	4.90	5.10	5.29	5.48	5.67	5.87
21.0	3551	4.56	4.65	4.85	5.04	5.24	5.44	5.62	5.82	6.02
21.2	3559	4.70	4.80	4.99	5.19	5.39	5.58	5.77	5.96	6.16
21.4	3566	4.84	4.94	5.14	5.33	5.53	5.72	5.91	6.11	6.30
21.6	3574	4.99	5.09	5.28	5.47	5.67	5.87	6.06	6.25	6.44
21.8	3582	5.13	5.23	5.43	5.61	5.82	6.01	6.20	6.39	6.59

<sup>1</sup> Rearranged from the table of B. H. St. John, which is based upon the data of Doroshevskii and Dvorzhanskii, *J. Russ. Phys. Chem. Soc.*, 40, 101 (1908). The scale readings were converted into refractive indices by using the formula  $n_D = 1.327338 + 0.00039347X + 0.00000020446X^2$ .

<sup>2</sup> The scale readings refer only to the scale of arbitrary units proposed by Pulfrich, *Z. angew. Chem.*, p. 1168, 1899. According to this scale, 14.5 = 1.33300, 50.0 = 1.34650, and 100.0 = 1.36464. If the immersion refractometer used is calibrated to another arbitrary scale, the readings must be converted into refractive indices before the table is used to determine the percentage of alcohol.

44.24

*Alcohol table.*—Continued.

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
22.0	1.33590	5.27	5.37	5.57	5.76	5.96	6.15	6.34	6.54	6.73
22.2	3597	5.41	5.51	5.71	5.90	6.11	6.29	6.49	6.68	6.87
22.4	3605	5.56	5.65	5.85	6.05	6.25	6.43	6.63	6.82	7.01
22.6	3613	5.70	5.80	6.00	6.19	6.39	6.57	6.77	6.96	7.16
22.8	3620	5.85	5.94	6.14	6.33	6.53	6.71	6.91	7.10	7.31
23.0	3628	5.99	6.08	6.28	6.47	6.67	6.86	7.06	7.24	7.45
23.2	3636	6.13	6.22	6.42	6.61	6.81	7.00	7.20	7.39	7.59
23.4	3643	6.27	6.36	6.56	6.75	6.95	7.14	7.34	7.53	7.73
23.6	3651	6.41	6.50	6.70	6.90	7.09	7.28	7.48	7.67	7.87
23.8	3659	6.55	6.64	6.85	7.04	7.23	7.42	7.62	7.81	8.00
24.0	3666	6.69	6.78	6.99	7.18	7.38	7.56	7.76	7.95	8.14
24.2	3674	6.83	6.92	7.13	7.32	7.52	7.70	7.90	8.09	8.28
24.4	3682	6.97	7.06	7.27	7.46	7.66	7.84	8.04	8.23	8.42
24.6	3689	7.11	7.20	7.41	7.60	7.80	7.98	8.17	8.37	8.55
24.8	3697	7.25	7.35	7.55	7.74	7.93	8.12	8.31	8.51	8.69
25.0	3705	7.39	7.49	7.68	7.88	8.06	8.26	8.45	8.64	8.84
25.2	3712	7.53	7.63	7.82	8.01	8.20	8.40	8.59	8.78	8.98
25.4	3720	7.66	7.76	7.95	8.14	8.34	8.54	8.73	8.92	9.12
25.6	3728	7.80	7.90	8.09	8.28	8.48	8.68	8.86	9.06	9.26
25.8	3735	7.94	8.03	8.22	8.42	8.62	8.82	9.00	9.20	9.39
26.0	3743	8.07	8.16	8.36	8.55	8.75	8.95	9.14	9.34	9.53
26.2	3751	8.21	8.30	8.50	8.69	8.89	9.09	9.28	9.48	9.67
26.4	3758	8.34	8.44	8.63	8.82	9.03	9.22	9.42	9.61	9.81
26.6	3766	8.48	8.57	8.77	8.96	9.16	9.36	9.55	9.75	9.95
26.8	3774	8.62	8.71	8.91	9.10	9.30	9.49	9.69	9.89	10.09
27.0	3781	8.75	8.85	9.05	9.23	9.44	9.63	9.83	10.03	10.23
27.2	3789	8.89	8.98	9.18	9.37	9.58	9.76	9.97	10.17	10.37
27.4	3796	9.02	9.12	9.32	9.51	9.71	9.90	10.10	10.31	10.51
27.6	3804	9.16	9.26	9.45	9.65	9.85	10.03	10.24	10.45	10.65
27.8	3812	9.29	9.39	9.59	9.79	9.98	10.17	10.38	10.58	10.79
28.0	3820	9.43	9.53	9.72	9.92	10.12	10.31	10.51	10.72	10.93
28.2	3827	9.57	9.66	9.86	10.06	10.25	10.45	10.65	10.86	11.06
28.4	3835	9.70	9.80	9.99	10.19	10.39	10.59	10.79	11.00	11.20
28.6	3842	9.84	9.93	10.13	10.32	10.52	10.72	10.93	11.13	11.33
28.8	3850	9.97	10.07	10.26	10.46	10.66	10.86	11.06	11.27	11.47
29.0	3858	10.10	10.19	10.40	10.59	10.79	11.00	11.20	11.40	11.61
29.2	3865	10.24	10.33	10.52	10.73	10.93	11.13	11.33	11.54	11.75
29.4	3873	10.36	10.46	10.66	10.86	11.06	11.27	11.47	11.67	11.88
29.6	3881	10.50	10.59	10.79	10.99	11.20	11.39	11.60	11.81	12.01
29.8	3888	10.63	10.72	10.93	11.12	11.33	11.53	11.74	11.94	12.15
30.0	3896	10.76	10.86	11.05	11.26	11.46	11.66	11.87	12.08	12.29
30.2	3904	10.89	10.99	11.18	11.38	11.59	11.79	12.00	12.21	12.42
30.4	3911	11.02	11.12	11.31	11.51	11.72	11.93	12.13	12.34	12.56
30.6	3919	11.15	11.25	11.44	11.64	11.85	12.06	12.27	12.48	12.70
30.8	3926	11.28	11.38	11.58	11.78	11.99	12.19	12.40	12.61	12.84

*Alcohol table.*—Continued.

44.24

SCALE. READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
31.0	1.33934	11.41	11.51	11.71	11.91	12.12	12.32	12.54	12.75	12.97
31.2	3942	11.54	11.64	11.84	12.04	12.25	12.46	12.67	12.89	13.11
31.4	3949	11.66	11.77	11.97	12.17	12.38	12.59	12.81	13.02	13.24
31.6	3957	11.79	11.90	12.10	12.30	12.51	12.72	12.94	13.15	13.37
31.8	3964	11.92	12.03	12.23	12.43	12.64	12.85	13.07	13.29	13.51
32.0	3972	12.05	12.15	12.36	12.57	12.78	12.99	13.20	13.42	13.64
32.2	3980	12.18	12.28	12.49	12.70	12.91	13.12	13.34	13.55	13.77
32.4	3987	12.31	12.40	12.62	12.83	13.04	13.25	13.47	13.69	13.91
32.6	3995	12.43	12.54	12.75	12.96	13.17	13.38	13.60	13.82	14.04
32.8	4002	12.56	12.67	12.88	13.09	13.30	13.51	13.73	13.95	14.17
33.0	4010	12.69	12.79	13.01	13.22	13.43	13.64	13.86	14.09	14.31
33.2	4018	12.82	12.92	13.13	13.35	13.56	13.78	13.99	14.22	14.44
33.4	4025	12.95	13.05	13.26	13.48	13.69	13.91	14.13	14.35	14.58
33.6	4033	13.08	13.18	13.39	13.61	13.82	14.04	14.26	14.48	14.71
33.8	4040	13.20	13.30	13.52	13.74	13.95	14.17	14.39	14.62	14.85
34.0	4048	13.33	13.43	13.64	13.86	14.08	14.30	14.52	14.75	14.98
34.2	4056	13.45	13.56	13.77	13.99	14.21	14.43	14.65	14.88	15.11
34.4	4063	13.58	13.68	13.90	14.12	14.34	14.57	14.78	15.01	15.25
34.6	4071	13.70	13.81	14.02	14.25	14.47	14.70	14.91	15.14	15.38
34.8	4078	13.83	13.94	14.14	14.37	14.59	14.83	15.05	15.28	15.51
35.0	4086	13.96	14.06	14.27	14.50	14.72	14.96	15.18	15.41	15.65
35.2	4094	14.08	14.19	14.39	14.62	14.85	15.09	15.31	15.54	15.78
35.4	4101	14.21	14.31	14.52	14.75	14.97	15.22	15.44	15.67	15.91
35.6	4109	14.33	14.44	14.65	14.87	15.10	15.34	15.56	15.80	16.05
35.8	4116	14.46	14.56	14.78	15.00	15.23	15.47	15.69	15.93	16.18
36.0	4124	14.58	14.69	14.90	15.13	15.35	15.59	15.82	16.06	16.31
36.2	4131	14.71	14.81	15.03	15.25	15.48	15.72	15.95	16.19	16.44
36.4	4139	14.83	14.94	15.16	15.38	15.61	15.85	16.08	16.32	16.56
36.6	4146	14.96	15.06	15.28	15.51	15.73	15.97	16.21	16.45	16.69
36.8	4154	15.08	15.19	15.41	15.63	15.86	16.10	16.34	16.58	16.82
37.0	4162	15.20	15.31	15.53	15.76	15.99	16.23	16.47	16.71	16.95
37.2	4169	15.33	15.44	15.66	15.89	16.11	16.35	16.60	16.84	17.08
37.4	4177	15.45	15.56	15.79	16.01	16.24	16.48	16.72	16.97	17.21
37.6	4184	15.57	15.69	15.91	16.14	16.37	16.61	16.85	17.09	17.34
37.8	4192	15.70	15.81	16.04	16.26	16.49	16.73	16.98	17.22	17.46
38.0	4199	15.82	15.94	16.16	16.39	16.62	16.86	17.11	17.35	17.59
38.2	4207	15.94	16.06	16.29	16.51	16.75	16.99	17.23	17.47	17.72
38.4	4215	16.07	16.18	16.41	16.64	16.87	17.11	17.36	17.60	17.85
38.6	4222	16.19	16.31	16.53	16.76	17.00	17.24	17.48	17.73	17.97
38.8	4230	16.31	16.43	16.66	16.89	17.13	17.36	17.61	17.85	18.10
39.0	4237	16.44	16.55	16.78	17.01	17.25	17.49	17.74	17.98	18.23
39.2	4245	16.56	16.67	16.91	17.14	17.38	17.62	17.86	18.11	18.35
39.4	4252	16.68	16.80	17.03	17.26	17.50	17.74	17.99	18.23	18.48
39.6	4260	16.80	16.92	17.15	17.39	17.63	17.87	18.11	18.36	18.61
39.8	4267	16.93	17.04	17.28	17.51	17.75	17.99	18.24	18.48	18.73

44.24

*Alcohol table.*—Continued.

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
40.0	1.34275	17.05	17.16	17.40	17.63	17.88	18.12	18.36	18.61	18.86
40.2	4282	17.17	17.29	17.52	17.76	18.00	18.24	18.49	18.74	18.99
40.4	4290	17.29	17.41	17.64	17.88	18.12	18.37	18.61	18.86	19.11
40.6	4298	17.41	17.53	17.77	18.01	18.25	18.49	18.74	18.99	19.24
40.8	4305	17.54	17.65	17.89	18.13	18.37	18.61	18.86	19.11	19.37
41.0	4313	17.66	17.77	18.01	18.25	18.49	18.74	18.99	19.24	19.49
41.2	4320	17.78	17.90	18.13	18.37	18.62	18.86	19.11	19.36	19.62
41.4	4328	17.90	18.03	18.26	18.50	18.74	18.99	19.24	19.49	19.75
41.6	4335	18.02	18.14	18.38	18.62	18.86	19.11	19.36	19.61	19.87
41.8	4343	18.14	18.26	18.50	18.74	18.99	19.23	19.48	19.74	20.00
42.0	4350	18.27	18.38	18.62	18.87	19.11	19.36	19.61	19.86	20.13
42.2	4358	18.39	18.50	18.74	18.99	19.23	19.48	19.73	19.99	20.25
42.4	4365	18.51	18.62	18.87	19.11	19.36	19.60	19.86	20.11	20.38
42.6	4373	18.63	18.75	18.99	19.23	19.48	19.72	19.98	20.24	20.50
42.8	4380	18.75	18.87	19.11	19.36	19.60	19.85	20.10	20.36	20.63
43.0	4388	18.87	18.99	19.23	19.48	19.72	19.97	20.23	20.49	20.75
43.2	4395	18.99	19.11	19.35	19.60	19.85	20.09	20.35	20.61	20.88
43.4	4403	19.11	19.23	19.47	19.72	19.97	20.21	20.47	20.74	21.01
43.6	4410	19.23	19.35	19.59	19.85	20.09	20.34	20.60	20.86	21.13
43.8	4418	19.35	19.47	19.72	19.97	20.21	20.46	20.72	20.99	21.25
44.0	4426	19.46	19.59	19.84	20.09	20.34	20.58	20.84	21.11	21.38
44.2	4433	19.58	19.71	19.96	20.21	20.46	20.71	20.96	21.23	21.50
44.4	4440	19.70	19.83	20.08	20.33	20.58	20.83	21.09	21.36	21.63
44.6	4448	19.82	19.95	20.20	20.45	20.70	20.95	21.21	21.48	21.75
44.8	4456	19.94	20.07	20.32	20.58	20.82	21.07	21.33	21.60	21.88
45.0	4463	20.06	20.18	20.44	20.70	20.95	21.19	21.45	21.73	22.00
45.2	4470	20.18	20.30	20.56	20.82	21.07	21.31	21.58	21.85	22.13
45.4	4478	20.29	20.42	20.68	20.94	21.19	21.43	21.70	21.98	22.25
45.6	4486	20.41	20.54	20.80	21.06	21.31	21.55	21.82	22.10	22.38
45.8	4493	20.53	20.66	20.92	21.18	21.43	21.67	21.94	22.23	22.51
46.0	4500	20.65	20.78	21.04	21.30	21.54	21.79	22.07	22.35	22.64
46.2	4508	20.76	20.89	21.16	21.42	21.66	21.91	22.19	22.48	22.76
46.4	4516	20.88	21.01	21.28	21.54	21.78	22.03	22.32	22.61	22.89
46.6	4523	21.00	21.13	21.40	21.66	21.90	22.16	22.44	22.73	23.02
46.8	4530	21.12	21.25	21.52	21.78	22.02	22.28	22.57	22.86	23.15
47.0	4538	21.24	21.37	21.64	21.90	22.15	22.41	22.69	22.99	23.28
47.2	4545	21.36	21.49	21.76	22.02	22.27	22.53	22.82	23.12	23.41
47.4	4553	21.48	21.61	21.88	22.15	22.39	22.66	22.94	23.24	23.54
47.6	4560	21.60	21.73	22.00	22.27	22.51	22.78	23.07	23.37	23.67
47.8	4568	21.72	21.85	22.12	22.39	22.64	22.91	23.20	23.50	23.80
48.0	4575	21.84	21.97	22.24	22.51	22.76	23.03	23.32	23.63	23.93
48.2	4583	21.96	22.09	22.36	22.63	22.88	23.16	23.45	23.76	24.06
48.4	4590	22.08	22.21	22.48	22.75	23.01	23.28	23.58	23.89	24.19
48.6	4598	22.20	22.33	22.60	22.87	23.13	23.41	23.71	24.02	24.32
48.8	4605	22.32	22.45	22.72	22.99	23.26	23.54	23.83	24.14	24.45



*Alcohol table.*—Continued.

44.24

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
49.0	1.34613	22.44	22.57	22.84	23.12	23.38	23.66	23.96	24.27	24.59
49.2	4620	22.56	22.69	22.96	23.24	23.51	23.79	24.09	24.40	24.72
49.4	4628	22.68	22.81	23.08	23.36	23.63	23.92	24.22	24.53	24.85
49.6	4635	22.80	22.93	23.21	23.48	23.76	24.04	24.35	24.66	24.98
49.8	4643	22.92	23.05	23.33	23.61	23.88	24.17	24.48	24.79	25.11
50.0	4650	23.04	23.17	23.45	23.73	24.01	24.30	24.61	24.92	25.25
50.2	4658	23.16	23.30	23.57	23.85	24.13	24.43	24.74	25.05	25.38
50.4	4665	23.28	23.42	23.69	23.98	24.26	24.56	24.86	25.18	25.51
50.6	4672	23.40	23.54	23.81	24.10	24.38	24.69	24.99	25.32	25.65
50.8	4680	23.51	23.66	23.93	24.22	24.51	24.81	25.12	25.45	25.78
51.0	4687	23.63	23.78	24.05	24.35	24.64	24.94	25.25	25.58	25.91
51.2	4695	23.75	23.90	24.18	24.47	24.76	25.07	25.38	25.71	26.05
51.4	4702	23.87	24.02	24.30	24.59	24.89	25.20	25.51	25.84	26.18
51.6	4710	23.99	24.14	24.42	24.72	25.01	25.33	25.64	25.97	26.32
51.8	4717	24.11	24.26	24.54	24.84	25.14	25.46	25.77	26.11	26.45
52.0	4724	24.23	24.38	24.66	24.96	25.27	25.58	25.90	26.24	26.59
52.2	4732	24.36	24.50	24.79	25.09	25.39	25.71	26.03	26.37	26.72
52.4	4740	24.48	24.62	24.91	25.21	25.52	25.84	26.16	26.51	26.86
52.6	4747	24.60	24.74	25.03	25.34	25.65	25.97	26.29	26.64	26.99
52.8	4754	24.72	24.86	25.15	25.46	25.77	26.10	26.42	26.77	27.13
53.0	4762	24.84	24.98	25.28	25.59	25.90	26.23	26.56	26.91	27.27
53.2	4769	24.96	25.10	25.40	25.71	26.03	26.35	26.69	27.04	27.40
53.4	4777	25.08	25.23	25.52	25.84	26.15	26.48	26.82	27.17	27.54
53.6	4784	25.20	25.35	25.65	25.96	26.28	26.61	26.95	27.31	27.67
53.8	4792	25.32	25.47	25.77	26.09	26.41	26.74	27.08	27.44	27.81
54.0	4799	25.44	25.59	25.90	26.22	26.54	26.87	27.21	27.58	27.95
54.2	4806	25.56	25.71	26.02	26.34	26.67	27.00	27.35	27.71	28.08
54.4	4814	25.68	25.84	26.14	26.47	26.79	27.13	27.48	27.85	28.22
54.6	4821	25.81	25.96	26.27	26.59	26.92	27.26	27.61	27.98	28.36
54.8	4829	25.93	26.08	26.39	26.72	27.05	27.39	27.75	28.11	28.49
55.0	4836	26.05	26.20	26.52	26.85	27.18	27.52	27.88	28.25	28.63
55.2	4844	26.17	26.32	26.64	26.97	27.31	27.65	28.01	28.38	28.77
55.4	4851	26.29	26.45	26.76	27.10	27.43	27.78	28.15	28.52	28.90
55.6	4858	26.41	26.57	26.89	27.23	27.55	27.92	28.28	28.65	29.04
55.8	4866	26.53	26.69	27.01	27.35	27.69	28.05	28.41	28.78	29.18
56.0	4873	26.65	26.81	27.14	27.48	27.82	28.18	28.54	28.92	29.31
56.2	4880	26.78	26.93	27.26	27.60	27.94	28.31	28.68	29.05	29.45
56.4	4888	26.90	27.05	27.38	27.73	28.07	28.44	28.81	29.19	29.58
56.6	4895	27.02	27.18	27.51	27.85	28.20	28.56	28.94	29.32	29.72
56.8	4903	27.14	27.30	27.63	27.98	28.33	28.69	29.07	29.46	29.86
57.0	4910	27.26	27.42	27.75	28.10	28.46	28.82	29.20	29.59	29.99
57.2	4918	27.38	27.54	27.88	28.23	28.59	28.95	29.34	29.73	30.13
57.4	4925	27.50	27.66	28.00	28.35	28.72	29.08	29.47	29.86	30.27
57.6	4932	27.62	27.79	28.13	28.48	28.85	29.21	29.60	30.00	30.41
57.8	4940	27.75	27.91	28.25	28.60	28.97	29.34	29.73	30.14	30.55

44.24

*Alcohol table.*—Continued.

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
58.0	1.34947	27.87	28.03	28.38	28.73	29.10	29.47	29.87	30.27	30.69
58.2	4954	27.99	28.15	28.50	28.86	29.23	29.60	29.99	30.41	30.83
58.4	4962	28.11	28.28	28.62	28.98	29.36	29.73	30.13	30.54	30.97
58.6	4969	28.23	28.40	28.75	29.11	29.48	29.86	30.26	30.68	31.11
58.8	4977	28.35	28.52	28.88	29.23	29.61	29.99	30.40	30.82	31.25
59.0	4984	28.47	28.64	29.00	29.36	29.74	30.13	30.53	30.95	31.40
59.2	4991	28.59	28.77	29.12	29.49	29.87	30.26	30.67	31.09	31.54
59.4	4999	28.71	28.89	29.25	29.61	29.99	30.39	30.81	31.23	31.68
59.6	5006	28.84	29.01	29.37	29.74	30.13	30.53	30.94	31.38	31.83
59.8	5014	28.96	29.13	29.50	29.87	30.26	30.66	31.08	31.52	31.97
60.0	5021	29.08	29.26	29.62	29.99	30.39	30.79	31.22	31.66	32.12
60.2	5028	29.20	29.38	29.74	30.12	30.52	30.93	31.36	31.80	32.27
60.4	5036	29.32	29.50	29.87	30.25	30.65	31.06	31.50	31.94	32.41
60.6	5043	29.45	29.63	29.99	30.38	30.78	31.20	31.64	32.09	32.56
60.8	5050	29.57	29.75	30.12	30.51	30.91	31.33	31.78	32.23	32.71
61.0	5058	29.69	29.87	30.25	30.64	31.05	31.47	31.92	32.38	32.86
61.2	5065	29.81	29.99	30.38	30.77	31.18	31.61	32.06	32.52	33.01
61.4	5073	29.93	30.12	30.50	30.90	31.32	31.74	32.20	32.67	33.16
61.6	5080	30.06	30.25	30.63	31.03	31.45	31.88	32.34	32.81	33.31
61.8	5087	30.18	30.37	30.76	31.16	31.59	32.01	32.49	32.96	33.46
62.0	5095	30.31	30.50	30.89	31.29	31.72	32.16	32.63	33.10	33.60
62.2	5102	30.43	30.63	31.01	31.43	31.86	32.30	32.77	33.25	33.75
62.4	5110	30.56	30.75	31.14	31.56	31.99	32.44	32.91	33.40	33.90
62.6	5117	30.69	30.88	31.28	31.69	32.13	32.58	33.06	33.55	34.05
62.8	5124	30.81	31.01	31.41	31.83	32.27	32.72	33.20	33.70	34.21
63.0	5132	30.94	31.14	31.54	31.96	32.41	32.87	33.35	33.84	34.36
63.2	5139	31.06	31.26	31.67	32.10	32.55	33.01	33.50	33.99	34.52
63.4	5146	31.19	31.39	31.80	32.23	32.69	33.15	33.64	34.15	34.67
63.6	5154	31.32	31.52	31.93	32.37	32.83	33.30	33.79	34.30	34.83
63.8	5161	31.45	31.65	32.07	32.51	32.97	33.44	33.93	34.45	34.98
64.0	5168	31.58	31.78	32.20	32.65	33.11	33.59	34.08	34.61	35.15
64.2	5176	31.70	31.91	32.34	32.79	33.25	33.73	34.23	34.76	35.31
64.4	5183	31.83	32.04	32.47	32.92	33.39	33.88	34.39	34.92	35.48
64.6	5190	31.96	32.17	32.60	33.06	33.53	34.02	34.54	35.07	35.64
64.8	5198	32.09	32.30	32.74	33.20	33.67	34.17	34.69	35.23	35.80
65.0	5205	32.22	32.43	32.87	33.34	33.82	34.32	34.84	35.39	35.97
65.2	5212	32.35	32.57	33.01	33.48	33.96	34.47	34.99	35.55	36.13
65.4	5220	32.48	32.70	33.15	33.62	34.10	34.61	35.15	35.71	36.30
65.6	5227	32.61	32.83	33.28	33.76	34.25	34.76	35.30	35.87	36.46
65.8	5234	32.75	32.96	33.42	33.90	34.40	34.91	35.46	36.02	36.63
66.0	5242	32.88	33.10	33.56	34.04	34.54	35.06	35.62	36.19	36.79
66.2	5249	33.01	33.23	33.70	34.18	34.69	35.22	35.77	36.35	36.96
66.4	5256	33.14	33.37	33.84	34.33	34.84	35.38	35.93	36.52	37.13
66.6	5264	33.28	33.51	33.98	34.47	34.99	35.53	36.09	36.68	37.30
66.8	5271	33.41	33.65	34.12	34.62	35.14	35.69	36.25	36.84	37.48

*Alcohol table.*—Continued.

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
67.0	1.35278	33.55	33.79	34.26	34.76	35.29	35.84	36.41	37.01	37.65
67.2	5286	33.69	33.92	34.41	34.91	35.44	36.00	36.57	37.18	37.83
67.4	5293	33.82	34.06	34.55	35.05	35.60	36.16	36.73	37.35	38.00
67.6	5300	33.96	34.20	34.69	35.20	35.75	36.32	36.90	37.52	38.18
67.8	5308	34.09	34.34	34.84	35.35	35.90	36.48	37.06	37.69	38.35
68.0	5315	34.23	34.48	34.98	35.50	36.05	36.63	37.23	37.86	38.53
68.2	5322	34.36	34.62	35.13	35.65	36.21	36.79	37.39	38.03	38.70
68.4	5329	34.50	34.76	35.27	35.80	36.37	36.95	37.56	38.21	38.88
68.6	5337	34.64	34.90	35.42	35.95	36.52	37.12	37.73	38.38	39.06
68.8	5344	34.77	35.04	35.57	36.10	36.68	37.28	37.90	38.56	39.24
69.0	5351	34.91	35.19	35.71	36.25	36.84	37.45	38.07	38.73	39.43
69.2	5359	35.04	35.33	35.86	36.41	36.99	37.61	38.24	38.90	39.61
69.4	5366	35.19	35.47	36.01	36.56	37.15	37.78	38.41	39.08	39.80
69.6	5373	35.34	35.62	36.16	36.72	37.32	37.94	38.58	39.26	39.98
69.8	5381	35.49	35.76	36.31	36.87	37.48	38.11	38.75	39.45	40.17
70.0	5388	35.64	35.91	36.46	37.02	37.64	38.28	38.92	39.63	40.35
70.2	5395	35.78	36.05	36.61	37.19	37.80	38.45	39.10	39.81	40.53
70.4	5402	35.93	36.20	36.76	37.35	37.97	38.61	39.28	39.99	40.72
70.6	5410	36.08	36.35	36.92	37.51	38.13	38.78	39.46	40.17	40.90
70.8	5417	36.23	36.50	37.07	37.67	38.30	38.95	39.64	40.35	41.08
71.0	5424	36.38	36.65	37.23	37.83	38.47	39.12	39.82	40.54	41.27
71.2	5432	36.53	36.80	37.39	37.99	38.63	39.30	40.00	40.72	41.46
71.4	5439	36.68	36.95	37.55	38.16	38.80	39.48	40.18	40.90	41.64
71.6	5446	36.83	37.11	37.71	38.32	38.97	39.65	40.36	41.08	41.83
71.8	5454	36.98	37.27	37.87	38.49	39.14	39.83	40.54	41.27	42.02
72.0	5461	37.13	37.42	38.02	38.65	39.31	40.01	40.72	41.45	42.21
72.2	5468	37.29	37.58	38.19	38.82	39.49	40.18	40.90	41.64	42.40
72.4	5475	37.44	37.73	38.35	38.98	39.66	40.36	41.08	41.82	42.58
72.6	5483	37.60	37.89	38.51	39.16	39.83	40.54	41.26	42.01	42.77
72.8	5490	37.75	38.05	38.67	39.33	40.01	40.71	41.45	42.19	42.96
73.0	5497	37.91	38.21	38.84	39.50	40.18	40.88	41.63	42.38	43.15
73.2	5504	38.06	38.37	39.00	39.67	40.36	41.06	41.81	42.56	43.33
73.4	5512	38.22	38.53	39.17	39.84	40.53	41.24	41.99	42.75	43.52
73.6	5519	38.38	38.69	39.34	40.02	40.70	41.42	42.17	42.93	43.70
73.8	5526	38.54	38.85	39.50	40.19	40.88	41.60	42.36	43.12	43.89
74.0	5533	38.70	39.01	39.67	40.36	41.05	41.78	42.54	43.31	44.08
74.2	5541	38.86	39.18	39.84	40.53	41.23	41.96	42.72	43.49	44.28
74.4	5548	39.02	39.34	40.01	40.71	41.41	42.15	42.91	43.68	44.48
74.6	5555	39.18	39.51	40.18	40.88	41.59	42.33	43.09	43.86	44.67
74.8	5563	39.35	39.68	40.35	41.05	41.77	42.51	43.28	44.05	44.87
75.0	5570	39.51	39.84	40.53	41.23	41.95	42.70	43.46	44.25	45.07
75.2	5577	39.68	40.01	40.70	41.41	42.13	42.88	43.65	44.44	45.29
75.4	5584	39.84	40.18	40.87	41.58	42.31	43.07	43.83	44.63	45.50
75.6	5592	40.01	40.35	41.04	41.76	42.49	43.25	44.02	44.83	45.71
75.8	5599	40.18	40.53	41.22	41.94	42.67	43.44	44.21	45.03	45.92

44.24

*Alcohol table.—Concluded.*

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
76.0	1.35606	40.35	40.70	41.40	42.12	42.85	43.63	44.41	45.24	46.12
76.2	5613	40.53	40.87	41.57	42.30	43.04	43.81	44.60	45.44	46.34
76.4	5621	40.70	41.04	41.75	42.48	43.22	44.00	44.80	45.65	46.56
76.6	5628	40.87	41.22	41.92	42.66	43.41	44.19	44.99	45.86	46.78
76.8	5635	41.04	41.39	42.10	42.84	43.60	44.38	45.19	46.07	47.00
77.0	5642	41.22	41.57	42.28	43.02	43.79	44.57	45.40	46.29	47.23
77.2	5650	41.39	41.74	42.46	43.20	43.97	44.76	45.60	46.51	47.45
77.4	5657	41.57	41.91	42.63	43.39	44.16	44.95	45.81	46.73	47.68
77.6	5664	41.75	42.09	42.81	43.57	44.35	45.15	46.01	46.95	47.91
77.8	5671	41.92	42.26	42.99	43.76	44.54	45.35	46.23	47.17	48.14
78.0	5678	42.09	42.43	43.17	43.94	44.73	45.56	46.45	47.40	48.37
78.2	5686	42.26	42.61	43.36	44.13	44.92	45.76	46.67	47.63	48.60
78.4	5693	42.44	42.78	43.54	44.32	45.12	45.96	46.89	47.85	48.84
78.6	5700	42.61	42.96	43.72	44.51	45.32	46.17	47.11	48.08	49.07
78.8	5707	42.78	43.14	43.91	44.70	45.52	46.39	47.34	48.31	49.31
79.0	5715	42.95	43.32	44.09	44.89	45.72	46.61	47.56	48.53	49.54
79.2	5722	43.13	43.50	44.28	45.08	45.92	46.83	47.79	48.76	49.77
79.4	5729	43.31	43.68	44.47	45.28	46.13	47.04	48.01	48.99	50.01
79.6	5736	43.49	43.86	44.65	45.48	46.34	47.26	48.23	49.22	50.24
79.8	5744	43.67	44.05	44.84	45.68	46.56	47.48	48.46	49.45	50.48
80.0	5751	43.85	44.24	45.04	45.88	46.77	47.70	48.68	49.68	50.71

*Percentages by weight corresponding to various percentages by volume at 44.25  
15.56°C. (60°F.) in mixtures of ethyl alcohol and water.<sup>1</sup>*

PER CENT ALCOHOL BY VOLUME AT 60° F.	PER CENT ALCOHOL BY WEIGHT	DIFFERENCES	PER CENT ALCOHOL BY VOLUME AT 60° F.	PER CENT ALCOHOL BY WEIGHT	DIFFERENCES
0	0.000	.....	50	42.487	.....
1	0.795	0.795	51	43.428	0.941
2	1.593	.798	52	44.374	.946
3	2.392	.799	53	45.326	.952
4	3.194	.802	54	46.283	.957
		.804			.962
5	3.998		55	47.245	
6	4.804	.806	56	48.214	.969
7	5.612	.808	57	49.187	.973
8	6.422	.810	58	50.167	.980
9	7.234	.812	59	51.154	.987
		.813			.993
10	8.047		60	52.147	
11	8.862	.815	61	53.146	.999
12	9.679	.817	62	54.152	1.006
13	10.497	.818	63	55.165	1.013
14	11.317	.820	64	56.184	1.019
		.821			1.024
15	12.138		65	57.208	
16	12.961	.823	66	58.241	1.033
17	13.786	.825	67	59.279	1.038
18	14.612	.826	68	60.325	1.046
19	15.440	.828	69	61.379	1.054
		.829			1.062
20	16.269		70	62.441	
21	17.100	.831	71	63.511	1.070
22	17.933	.833	72	64.588	1.077
23	18.768	.835	73	65.674	1.086
24	19.604	.836	74	66.768	1.094
		.839			1.102
25	20.443		75	67.870	
26	21.285	.842	76	68.982	1.112
27	22.127	.842	77	70.102	1.120
28	22.973	.846	78	71.234	1.132
29	23.820	.847	79	72.375	1.141
		.850			1.151
30	24.670		80	73.526	
31	25.524	.854	81	74.686	1.160
32	26.382	.858	82	75.858	1.172
33	27.242	.860	83	77.039	1.181
34	28.104	.862	84	78.233	1.194
		.867			1.208
35	28.971		85	79.441	
36	29.842	.871	86	80.662	1.221
37	30.717	.875	87	81.897	1.235
38	31.596	.879	88	83.144	1.247
39	32.478	.882	89	84.408	1.264
		.886			1.281
40	33.364		90	85.689	
41	34.254	.890	91	86.989	1.300
42	35.150	.896	92	88.310	1.321
43	36.050	.900	93	89.652	1.342
44	36.955	.905	94	91.025	1.373
		.910			1.398
45	37.865		95	92.423	
46	38.778	.913	96	93.851	1.428
47	39.697	.919	97	95.315	1.464
48	40.622	.925	98	96.820	1.505
49	41.551	.929	99	98.381	1.561
		.936			1.619
50	42.487	.....	100	100.000	.....

<sup>1</sup> Bureau of Standards Circular No. 19, p. 18 (1924).

44.26 Correction table for specific gravity of milk (Quevenne lactometer)<sup>1</sup>

LACTOMETER		TEMPERATURE (F)																			LACTOMETER	
		51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69		
20	19.3	19.4	19.4	19.5	19.6	19.7	19.8	19.9	19.9	20.0	20.1	20.2	20.2	20.3	20.4	20.5	20.6	20.7	20.9	21.0	20	
21	20.3	20.3	20.4	20.5	20.6	20.7	20.8	20.9	20.9	21.0	21.1	21.2	21.3	21.4	21.5	21.6	21.7	21.8	22.0	22.1	21	
22	21.3	21.3	21.4	21.5	21.6	21.7	21.8	21.9	21.9	22.0	22.1	22.2	22.3	22.4	22.5	22.6	22.7	22.8	23.0	23.1	22	
23	22.3	22.3	22.4	22.5	22.6	22.7	22.8	22.8	22.9	23.0	23.1	23.2	23.3	23.4	23.5	23.6	23.7	23.8	24.0	24.1	23	
24	23.3	23.3	23.4	23.5	23.6	23.6	23.7	23.8	23.9	24.0	24.1	24.2	24.3	24.4	24.5	24.6	24.7	24.9	25.0	25.1	24	
25	24.2	24.3	24.4	24.5	24.6	24.6	24.7	24.8	24.9	25.0	25.1	25.2	25.3	25.4	25.5	25.6	25.7	25.9	26.0	26.1	25	
26	25.2	25.2	25.3	25.4	25.5	25.6	25.7	25.8	25.9	26.0	26.1	26.2	26.3	26.5	26.6	26.7	26.8	27.0	27.1	27.2	26	
27	26.2	26.2	26.3	26.4	26.5	26.6	26.7	26.8	26.9	27.0	27.1	27.3	27.4	27.5	27.6	27.7	27.8	28.0	28.1	28.2	27	
28	27.1	27.2	27.3	27.4	27.5	27.6	27.7	27.8	27.9	28.0	28.1	28.3	28.4	28.5	28.6	28.7	28.8	29.0	29.1	29.2	28	
29	28.1	28.2	28.3	28.4	28.5	28.6	28.7	28.8	28.9	29.0	29.1	29.3	29.4	29.5	29.6	29.7	29.9	30.1	30.2	30.3	29	
30	29.1	29.1	29.2	29.3	29.4	29.6	29.7	29.8	29.9	30.0	30.1	30.3	30.4	30.5	30.7	30.8	30.9	31.1	31.2	31.3	30	
31	30.0	30.1	30.2	30.3	30.4	30.5	30.6	30.8	30.9	31.0	31.2	31.3	31.4	31.5	31.7	31.8	31.9	32.1	32.2	32.4	31	
32	31.0	31.1	31.2	31.3	31.4	31.5	31.6	31.7	31.9	32.0	32.2	32.3	32.5	32.6	32.7	32.9	33.0	33.2	33.3	33.4	32	
33	31.9	32.0	32.1	32.3	32.4	32.5	32.6	32.7	32.9	33.0	33.2	33.3	33.5	33.6	33.8	33.9	34.0	34.2	34.3	34.5	33	
34	32.9	33.0	33.1	33.2	33.3	33.5	33.6	33.7	33.9	34.0	34.2	34.3	34.5	34.6	34.8	34.9	35.0	35.2	35.3	35.5	34	
35	33.8	33.9	34.0	34.2	34.3	34.5	34.6	34.7	34.9	35.0	35.2	35.3	35.5	35.6	35.8	36.0	36.1	36.2	36.4	36.5	35	

<sup>1</sup> Paul G. Heineman, "Milk," p. 144. W. B. Saunders Co. (1921).

Table for determining total solids in milk from any given specific gravity and percentage of fat (Shaw and Eckles).  
(Results expressed as total solids, per cent)

PER- CENTAGE OF FAT	LACTOMETER READING AT 60° F. (QUEVENNE DEGREES).										
	26	27	28	29	30	31	32	33	34	35	36
2.00	8.90	9.15	9.40	9.65	9.90	10.15	10.40	10.66	10.91	11.16	11.41
2.05	8.96	9.21	9.46	9.71	9.96	10.21	10.46	10.72	10.97	11.22	11.47
2.10	9.02	9.27	9.52	9.77	10.02	10.27	10.52	10.78	11.03	11.28	11.53
2.15	9.08	9.33	9.58	9.83	10.08	10.33	10.58	10.84	11.09	11.34	11.59
2.20	9.14	9.39	9.64	9.89	10.14	10.39	10.64	10.90	11.15	11.40	11.65
2.25	9.20	9.45	9.70	9.95	10.20	10.45	10.70	10.96	11.21	11.46	11.71
2.30	9.26	9.51	9.76	10.01	10.26	10.51	10.76	11.02	11.27	11.52	11.77
2.35	9.32	9.57	9.82	10.07	10.32	10.57	10.82	11.08	11.33	11.58	11.83
2.40	9.38	9.63	9.88	10.13	10.38	10.63	10.88	11.14	11.39	11.64	11.89
2.45	9.44	9.69	9.94	10.19	10.44	10.69	10.94	11.20	11.45	11.70	11.95
2.50	9.50	9.75	10.00	10.25	10.50	10.75	11.00	11.26	11.51	11.76	12.01
2.55	9.56	9.81	10.06	10.31	10.56	10.81	11.06	11.32	11.57	11.82	12.07
2.60	9.62	9.87	10.12	10.37	10.62	10.87	11.12	11.38	11.63	11.88	12.13
2.65	9.68	9.93	10.18	10.43	10.68	10.93	11.18	11.44	11.69	11.94	12.19
2.70	9.74	9.99	10.24	10.49	10.74	10.99	11.24	11.50	11.75	12.00	12.25
2.75	9.80	10.05	10.30	10.55	10.80	11.05	11.31	11.56	11.81	12.06	12.31
2.80	9.86	10.11	10.36	10.61	10.86	11.11	11.37	11.62	11.87	12.12	12.37
2.85	9.92	10.17	10.42	10.67	10.92	11.17	11.43	11.68	11.93	12.18	12.43
2.90	9.98	10.23	10.48	10.73	10.98	11.23	11.49	11.74	11.99	12.24	12.49
2.95	10.04	10.29	10.54	10.79	11.04	11.30	11.55	11.80	12.05	12.30	12.55
3.00	10.10	10.35	10.60	10.85	11.10	11.36	11.61	11.86	12.11	12.36	12.61
3.05	10.16	10.41	10.66	10.91	11.17	11.42	11.67	11.92	12.17	12.42	12.68
3.10	10.22	10.47	10.72	10.97	11.23	11.48	11.73	11.98	12.23	12.48	12.74
3.15	10.28	10.53	10.78	11.03	11.29	11.54	11.79	12.04	12.29	12.55	12.80
3.20	10.34	10.59	10.84	11.09	11.35	11.60	11.85	12.10	12.35	12.61	12.86
3.25	10.40	10.65	10.90	11.16	11.41	11.66	11.91	12.16	12.42	12.67	12.92
3.30	10.46	10.71	10.96	11.22	11.47	11.72	11.97	12.22	12.48	12.73	12.98
3.35	10.52	10.77	11.03	11.28	11.53	11.78	12.03	12.28	12.54	12.79	13.04
3.40	10.58	10.83	11.09	11.34	11.59	11.84	12.09	12.34	12.60	12.85	13.10
3.45	10.64	10.89	11.15	11.40	11.65	11.90	12.15	12.40	12.66	12.91	13.16
3.50	10.70	10.95	11.21	11.46	11.71	11.96	12.21	12.46	12.72	12.97	13.22
3.55	10.76	11.02	11.27	11.52	11.77	12.02	12.27	12.52	12.78	13.03	13.28
3.60	10.82	11.08	11.33	11.58	11.83	12.08	12.33	12.58	12.84	13.09	13.34
3.65	10.88	11.14	11.39	11.64	11.89	12.14	12.39	12.64	12.90	13.15	13.40
3.70	10.94	11.20	11.45	11.70	11.95	12.20	12.45	12.70	12.96	13.21	13.46
3.75	11.00	11.26	11.51	11.76	12.01	12.26	12.51	12.76	13.02	13.27	13.52
3.80	11.06	11.32	11.57	11.82	12.07	12.32	12.57	12.82	13.08	13.33	13.58
3.85	11.12	11.38	11.63	11.88	12.13	12.38	12.63	12.88	13.14	13.39	13.64
3.90	11.18	11.44	11.69	11.94	12.19	12.44	12.69	12.94	13.20	13.45	13.70
3.95	11.24	11.50	11.75	12.00	12.25	12.50	12.75	13.00	13.26	13.51	13.77
4.00	11.30	11.56	11.81	12.06	12.31	12.56	12.81	13.06	13.32	13.57	13.83
4.05	11.36	11.62	11.87	12.12	12.37	12.62	12.87	13.12	13.38	13.63	13.89
4.10	11.42	11.68	11.93	12.18	12.43	12.68	12.93	13.18	13.44	13.69	13.95
4.15	11.48	11.74	11.99	12.24	12.49	12.74	12.99	13.25	13.50	13.76	14.01
4.20	11.54	11.80	12.05	12.30	12.55	12.80	13.05	13.31	13.56	13.82	14.07
4.25	11.60	11.86	12.11	12.36	12.61	12.86	13.12	13.37	13.62	13.88	14.13
4.30	11.66	11.92	12.17	12.42	12.67	12.92	13.18	13.43	13.68	13.94	14.19
4.35	11.72	11.98	12.23	12.48	12.73	12.98	13.24	13.49	13.74	14.00	14.25
4.40	11.78	12.04	12.29	12.54	12.79	13.04	13.30	13.55	13.80	14.06	14.31
4.45	11.84	12.10	12.35	12.60	12.85	13.10	13.36	13.61	13.86	14.12	14.37
4.50	11.90	12.16	12.41	12.66	12.91	13.16	13.42	13.67	13.92	14.18	14.43
4.55	11.97	12.22	12.47	12.72	12.97	13.22	13.48	13.73	13.98	14.24	14.49
4.60	12.03	12.28	12.53	12.78	13.03	13.28	13.54	13.79	14.04	14.30	14.55
4.65	12.09	12.34	12.59	12.84	13.09	13.34	13.60	13.85	14.10	14.36	14.61
4.70	12.15	12.40	12.65	12.90	13.15	13.40	13.66	13.91	14.16	14.42	14.67
4.75	12.21	12.46	12.71	12.96	13.21	13.46	13.72	13.97	14.22	14.48	14.73
4.80	12.27	12.52	12.77	13.02	13.27	13.52	13.78	14.03	14.28	14.54	14.79
4.85	12.33	12.58	12.83	13.08	13.33	13.58	13.84	14.09	14.34	14.60	14.85
4.90	12.39	12.64	12.89	13.14	13.39	13.64	13.90	14.15	14.40	14.66	14.91
4.95	12.45	12.70	12.95	13.20	13.45	13.70	13.96	14.21	14.46	14.72	14.97
5.00	12.51	12.76	13.01	13.26	13.51	13.76	14.02	14.27	14.52	14.78	15.03
5.05	12.57	12.82	13.07	13.32	13.57	13.83	14.08	14.33	14.58	14.84	15.09
5.10	12.63	12.88	13.13	13.38	13.63	13.89	14.14	14.39	14.64	14.90	15.15
5.15	12.69	12.94	13.19	13.44	13.69	13.95	14.20	14.45	14.70	14.96	15.21
5.20	12.75	13.00	13.25	13.50	13.75	14.01	14.26	14.51	14.76	15.02	15.27
5.25	12.81	13.06	13.31	13.56	13.81	14.07	14.32	14.57	14.82	15.08	15.33
5.30	12.87	13.12	13.37	13.62	13.87	14.13	14.38	14.63	14.88	15.14	15.39
5.35	12.93	13.18	13.43	13.68	13.93	14.19	14.44	14.70	14.95	15.20	15.45
5.40	12.99	13.24	13.49	13.74	14.00	14.25	14.50	14.76	15.01	15.26	15.51
5.45	13.05	13.30	13.55	13.80	14.06	14.31	14.56	14.82	15.07	15.32	15.57

**44.27** Table for determining total solids in milk from any given specific gravity and percentage of fat—Continued (Shaw and Eckles).

PER- CENTAGE OF FAT	LACTOMETER READING AT 60° F. (QUEVENNE DEGREES)										
	26	27	28	29	30	31	32	33	34	35	36
5.50	13.11	13.36	13.61	13.86	14.12	14.37	14.62	14.88	15.13	15.38	15.63
5.55	13.17	13.42	13.67	13.93	14.18	14.43	14.69	14.94	15.19	15.44	15.69
5.60	13.23	13.48	13.73	13.99	14.24	14.49	14.75	15.00	15.25	15.50	15.75
5.65	13.29	13.54	13.79	14.05	14.30	14.55	14.81	15.06	15.31	15.56	15.81
5.70	13.35	13.60	13.85	14.11	14.36	14.61	14.87	15.12	15.37	15.62	15.87
5.75	13.41	13.66	13.91	14.17	14.42	14.68	14.93	15.18	15.43	15.68	15.93
5.80	13.47	13.72	13.97	14.23	14.48	14.74	14.99	15.24	15.49	15.74	15.99
5.85	13.53	13.78	14.04	14.29	14.54	14.80	15.05	15.30	15.55	15.80	16.06
5.90	13.59	13.84	14.10	14.35	14.60	14.86	15.11	15.36	15.61	15.86	16.12
5.95	13.65	13.90	14.16	14.41	14.66	14.92	15.17	15.42	15.67	15.92	16.18
6.00	13.71	13.96	14.22	14.47	14.72	14.98	15.23	15.48	15.73	15.98	16.24
6.05	13.77	14.02	14.28	14.53	14.78	15.04	15.29	15.54	15.79	16.04	16.30
6.10	13.83	14.08	14.34	14.59	14.84	15.10	15.35	15.60	15.85	16.10	16.35
6.15	13.89	14.14	14.40	14.65	14.90	15.16	15.41	15.66	15.91	16.16	16.42
6.20	13.95	14.20	14.46	14.71	14.96	15.22	15.47	15.72	15.97	16.22	16.48
6.25	14.01	14.26	14.52	14.77	15.02	15.28	15.53	15.78	16.03	16.28	16.54
6.30	14.07	14.32	14.58	14.83	15.08	15.34	15.59	15.84	16.09	16.34	16.60
6.35	14.13	14.38	14.64	14.90	15.14	15.40	15.65	15.90	16.15	16.40	16.66
6.40	14.19	14.44	14.70	14.96	15.20	15.46	15.71	15.96	16.21	16.46	16.72
6.45	14.25	14.50	14.76	15.02	15.26	15.52	15.77	16.02	16.27	16.52	16.78
6.50	14.31	14.56	14.82	15.08	15.32	15.58	15.83	16.08	16.33	16.58	16.84
6.55	14.37	14.62	14.88	15.14	15.38	15.64	15.89	16.14	16.39	16.64	16.90
6.60	14.43	14.68	14.94	15.20	15.44	15.70	15.95	16.20	16.45	16.70	16.96
6.65	14.49	14.74	15.00	15.26	15.50	15.76	16.01	16.26	16.51	16.76	17.02
6.70	14.55	14.80	15.06	15.32	15.56	15.82	16.07	16.32	16.57	16.82	17.08
6.75	14.61	14.86	15.12	15.38	15.62	15.88	16.13	16.38	16.63	16.88	17.14
6.80	14.67	14.92	15.18	15.44	15.68	15.94	16.19	16.44	16.69	16.94	17.20
6.85	14.73	14.98	15.24	15.50	15.74	16.00	16.25	16.50	16.75	17.00	17.26
6.90	14.79	15.04	15.30	15.56	15.80	16.06	16.31	16.56	16.81	17.06	17.32
6.95	14.85	15.10	15.36	15.62	15.86	16.12	16.37	16.62	16.87	17.12	17.38

## PROPORTIONAL PARTS

LACTOMETER FRACTION	FRACTION TO BE ADDED TO TOTAL SOLIDS	LACTOMETER FRACTION	FRACTION TO BE ADDED TO TOTAL SOLIDS	LACTOMETER FRACTION	FRACTION TO BE ADDED TO TOTAL SOLIDS
0.1	0.03	0.4	0.10	0.7	0.18
.2	.05	.5	.13	.8	.20
.3	.08	.6	.15	.9	.23

The table giving Proportional Parts shows the amount to be added when lactometer readings are in whole numbers and decimals.



*For determining added water in milk by means of the freezing-point depression (based on Winter's table<sup>1</sup>).* 44.28

(For practical purposes the added water results may be expressed to the nearest decimal.)

FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME	FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME	FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME
0.550	0.00	0.505	8.18	0.460	16.36
.549	0.18	.504	8.36	.459	16.54
.548	0.36	.503	8.54	.458	16.73
.547	0.54	.502	8.73	.457	16.91
.546	0.73	.501	8.91	.456	17.09
.545	0.91	.500	9.09	.455	17.27
.544	1.09	.499	9.27	.454	17.45
.543	1.27	.498	9.45	.453	17.64
.542	1.45	.497	9.64	.452	17.82
.541	1.63	.496	9.82	.451	18.00
.540	1.82	.495	10.00	.450	18.18
.539	2.00	.494	10.18	.449	18.36
.538	2.18	.493	10.36	.448	18.54
.537	2.36	.492	10.54	.447	18.73
.536	2.54	.491	10.72	.446	18.91
.535	2.72	.490	10.91	.445	19.09
.534	2.91	.489	11.09	.444	19.27
.533	3.09	.488	11.27	.443	19.45
.532	3.27	.487	11.45	.442	19.64
.531	3.45	.486	11.64	.441	19.82
.530	3.64	.485	11.82	.440	20.00
.529	3.82	.484	12.00	.439	20.18
.528	4.00	.483	12.18	.438	20.36
.527	4.18	.482	12.36	.437	20.54
.526	4.36	.481	12.54	.436	20.73
.525	4.54	.480	12.73	.435	20.91
.524	4.73	.479	12.91	.434	21.09
.523	4.91	.478	13.09	.433	21.27
.522	5.09	.477	13.27	.432	21.45
.521	5.27	.476	13.45	.431	21.64
.520	5.45	.475	13.64	.430	21.82
.519	5.63	.474	13.82	.429	22.00
.518	5.82	.473	14.00	.428	22.18
.517	6.00	.472	14.18	.427	22.36
.516	6.18	.471	14.37	.426	22.54
.515	6.36	.470	14.54	.425	22.73
.514	6.54	.469	14.73	.424	22.91
.513	6.73	.468	14.91	.423	23.09
.512	6.91	.467	15.09	.422	23.27
.511	7.09	.466	15.27	.421	23.45
.510	7.27	.465	15.45	.420	23.64
.509	7.45	.464	15.63	.419	23.82
.508	7.64	.463	15.82	.418	24.00
.507	7.82	.462	16.00	.417	24.18
.506	8.00	.461	16.18	.416	24.36

<sup>1</sup> Chem. News, 110, 283 (1914).

**44.28** *For determining added water in milk by means of the freezing-point depression (based on Winter's table).—Concluded.*

FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME	FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME	FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME
0.415	24.54	0.390	29.09	0.365	33.64
.414	24.73	.389	29.27	.364	33.82
.413	24.91	.388	29.45	.363	34.00
.412	25.09	.387	29.64	.362	34.18
.411	25.27	.386	29.82	.361	34.36
.410	25.45	.385	30.00	.360	34.54
.409	25.64	.384	30.18	.359	34.73
.408	25.82	.383	30.36	.358	34.91
.407	26.00	.382	30.54	.357	35.09
.406	26.18	.381	30.73	.356	35.27
.405	26.36	.380	30.91	.355	35.45
.404	26.54	.379	31.09	.354	35.64
.403	26.73	.378	31.27	.353	35.82
.402	26.91	.377	31.45	.352	36.00
.401	27.09	.376	31.64	.351	36.18
.400	27.27	.375	31.82	.350	36.36
.399	27.45	.374	32.00		
.398	27.64	.373	32.18		
.397	27.82	.372	32.36		
.396	28.00	.371	32.54		
.395	28.18	.370	32.73		
.394	28.36	.369	32.91		
.393	28.54	.368	33.09		
.392	28.73	.367	33.27		
.391	28.91	.366	33.45		

*Density of carbon dioxide (Parr).<sup>1</sup>*

44.29

(Weight in milligrams of 1 ml of carbon dioxide at 700–770 mm pressure and 10–30°C. Corrected for aqueous vapor and barometer readings on glass scale. Calculated from 1.976 equals weight of liter of CO<sub>2</sub> at 0°C., 760 mm pressure and 41° latitude.)

mm.	10°	11°	12°	13°	14°	15°	16°	17°	18°	19°
700	1.7288	1.7201	1.7113	1.7020	1.6927	1.6833	1.6799	1.6716	1.6632	1.6547
702	.7338	.7252	.7164	.7072	.6980	.6914	.6848	.6765	.6680	.6595
704	.7388	.7302	.7215	.7124	.7033	.6965	.6897	.6813	.6729	.6644
706	.7438	.7353	.7266	.7176	.7086	.7016	.6946	.6862	.6778	.6692
708	.7488	.7403	.7317	.7228	.7139	.7067	.6995	.6911	.6826	.6741
710	.7538	.7453	.7368	.7280	.7192	.7118	.7044	.6960	.6874	.6789
712	.7588	.7504	.7419	.7332	.7245	.7169	.7092	.7008	.6922	.6837
714	.7638	.7555	.7470	.7384	.7298	.7220	.7141	.7057	.6970	.6886
716	.7688	.7605	.7521	.7436	.7351	.7271	.7190	.7106	.7019	.6934
718	.7738	.7656	.7572	.7488	.7404	.7322	.7239	.7154	.7068	.6983
720	.7788	.7706	.7623	.7540	.7457	.7373	.7288	.7203	.7117	.7031
722	.7838	.7756	.7673	.7590	.7506	.7422	.7337	.7252	.7166	.7079
724	.7888	.7806	.7723	.7639	.7555	.7471	.7386	.7301	.7215	.7128
726	.7938	.7856	.7773	.7689	.7605	.7520	.7435	.7349	.7263	.7176
728	.7988	.7905	.7822	.7738	.7654	.7569	.7484	.7398	.7312	.7225
730	.8038	.7955	.7872	.7788	.7703	.7618	.7533	.7447	.7360	.7273
732	.8089	.8005	.7921	.7837	.7752	.7667	.7582	.7496	.7409	.7321
734	.8139	.8055	.7971	.7887	.7802	.7717	.7631	.7545	.7458	.7370
736	.8189	.8105	.8021	.7936	.7851	.7766	.7680	.7593	.7506	.7418
738	.8239	.8155	.8071	.7986	.7901	.7815	.7729	.7642	.7555	.7467
740	.8288	.8204	.8120	.8035	.7950	.7864	.7778	.7691	.7603	.7515
742	.8338	.8254	.8170	.8085	.7999	.7913	.7827	.7740	.7652	.7564
744	.8388	.8304	.8219	.8134	.8048	.7962	.7875	.7788	.7700	.7612
746	.8439	.8354	.8269	.8184	.8098	.8011	.7924	.7837	.7749	.7661
748	.8489	.8404	.8319	.8233	.8147	.8060	.7973	.7886	.7798	.7709
750	.8539	.8454	.8368	.8282	.8196	.8109	.8022	.7934	.7846	.7757
752	.8589	.8504	.8418	.8332	.8246	.8159	.8072	.7984	.7895	.7806
754	.8639	.8554	.8468	.8382	.8295	.8208	.8120	.8032	.7944	.7854
756	.8689	.8603	.8517	.8431	.8344	.8257	.8169	.8081	.7992	.7902
758	.8739	.8653	.8567	.8481	.8394	.8306	.8218	.8130	.8041	.7951
760	.8789	.8703	.8617	.8530	.8443	.8355	.8267	.8178	.8089	.7999
762	.8839	.8753	.8667	.8580	.8492	.8404	.8316	.8227	.8138	.8048
764	.8890	.8803	.8716	.8629	.8541	.8453	.8365	.8276	.8187	.8096
766	.8940	.8853	.8766	.8679	.8591	.8503	.8414	.8325	.8235	.8144
768	.8990	.8903	.8816	.8728	.8640	.8552	.8463	.8374	.8284	.8193
770	.9040	.8953	.8865	.8777	.8689	.8601	.8512	.8422	.8332	.8241

<sup>1</sup> *J. Am. Chem. Soc.*, 31, 237 (1919). The values of 700–718 mm were calculated by formula given by Parr.

## 44.29

*Density of carbon dioxide (Parr).—Concluded.*

20°	21°	22°	23°	24°	25°	26°	27°	28°	29°	30°	mm.
1.6462	1.6370	1.6278	1.6195	1.6112	1.6021	1.5930	1.5837	1.5744	1.5649	1.5554	700
.6510	.6419	.6327	.6243	.6160	.6068	.5977	.5884	.5791	.5696	.5600	702
.6558	.6467	.6376	.6292	.6207	.6116	.6025	.5931	.5838	.5742	.5647	704
.6607	.6516	.6425	.6340	.6254	.6163	.6072	.5979	.5885	.5789	.5693	706
.6655	.6564	.6474	.6388	.6302	.6211	.6119	.6026	.5932	.5836	.5740	708
.6703	.6613	.6522	.6436	.6350	.6258	.6166	.6073	.5978	.5882	.5786	710
.6751	.6662	.6571	.6485	.6397	.6305	.6214	.6120	.6025	.5929	.5832	712
.6799	.6710	.6620	.6533	.6444	.6353	.6261	.6167	.6072	.5976	.5879	714
.6848	.6759	.6670	.6581	.6492	.6400	.6308	.6215	.6119	.6023	.5925	716
.6896	.6807	.6718	.6629	.6540	.6448	.6356	.6262	.6166	.6069	.5972	718
.6944	.6856	.6767	.6678	.6587	.6495	.6403	.6309	.6213	.6116	.6018	720
.6992	.6904	.6815	.6726	.6635	.6543	.6450	.6356	.6260	.6163	.6065	722
.7041	.6953	.6863	.6773	.6682	.6590	.6497	.6403	.6307	.6210	.6111	724
.7089	.7001	.6911	.6821	.6730	.6638	.6544	.6450	.6354	.6256	.6157	726
.7137	.7049	.6959	.6869	.6778	.6685	.6591	.6497	.6401	.6303	.6204	728
.7185	.7097	.7007	.6917	.6825	.6732	.6638	.6544	.6448	.6350	.6251	730
.7233	.7145	.7055	.6964	.6872	.6779	.6685	.6591	.6494	.6396	.6297	732
.7282	.7193	.7103	.7012	.6920	.6827	.6733	.6638	.6541	.6443	.6343	734
.7330	.7241	.7151	.7060	.6968	.6875	.6780	.6685	.6589	.6490	.6390	736
.7378	.7289	.7199	.7107	.7015	.6922	.6827	.6732	.6635	.6537	.6437	738
.7426	.7337	.7247	.7155	.7063	.6969	.6874	.6778	.6681	.6583	.6483	740
.7475	.7385	.7295	.7203	.7111	.7017	.6922	.6826	.6729	.6630	.6530	742
.7523	.7433	.7342	.7250	.7158	.7064	.6969	.6873	.6776	.6677	.6577	744
.7571	.7481	.7390	.7298	.7206	.7112	.7016	.6920	.6822	.6723	.6623	746
.7619	.7529	.7438	.7346	.7253	.7159	.7063	.6967	.6869	.6770	.6670	748
.7667	.7577	.7486	.7394	.7301	.7206	.7110	.7014	.6916	.6817	.6716	750
.7716	.7625	.7534	.7441	.7348	.7254	.7158	.7061	.6963	.6864	.6763	752
.7764	.7673	.7582	.7489	.7396	.7301	.7205	.7108	.7010	.6910	.6809	754
.7812	.7721	.7630	.7537	.7443	.7348	.7252	.7155	.7057	.6957	.6856	756
.7861	.7770	.7678	.7585	.7491	.7396	.7300	.7202	.7104	.7004	.6903	758
.7909	.7818	.7725	.7632	.7538	.7443	.7347	.7249	.7150	.7050	.6949	760
.7957	.7866	.7773	.7680	.7586	.7490	.7394	.7296	.7197	.7097	.6996	762
.8005	.7914	.7821	.7728	.7633	.7538	.7441	.7343	.7244	.7144	.7042	764
.8053	.7962	.7869	.7776	.7681	.7585	.7488	.7390	.7291	.7191	.7089	766
.8102	.8010	.7917	.7823	.7728	.7633	.7535	.7437	.7338	.7237	.7135	768
.8150	.8058	.7965	.7871	.7776	.7680	.7582	.7484	.7385	.7284	.7182	770

**Correction factors for the gasometric determination of carbon dioxide.<sup>1</sup> 44.30**  
**(Based on sample weighing 1.7000 grams.)**

(Multiply the number of ml of gas evolved from 1.7000 grams of sample by the factor that corresponds with existing atmospheric conditions and divide by ten to obtain percentage of carbon dioxide by weight in sample.)

°C.	15.0°	15.5°	16.0°	16.5°	17.0°	17.5°	18.0°	18.5°	
mm.									inches
700	0.99194	0.99006	0.98818	0.98573	0.98329	0.98082	0.97835	0.97585	27.56
702	0.99494	0.99300	0.99106	0.98862	0.98618	0.98368	0.98118	0.97868	27.64
704	0.99794	0.99544	0.99394	0.99147	0.98900	0.98653	0.98406	0.98156	27.72
706	1.00094	0.99886	0.99682	0.99435	0.99188	0.98941	0.98694	0.98406	27.80
708	1.00394	1.00183	0.99971	0.99723	0.99476	0.99226	0.98976	0.98726	27.87
710	1.00694	1.00477	1.00259	1.00012	0.99765	0.99512	0.99259	0.99009	27.95
712	1.00994	1.00767	1.00541	1.00294	1.00047	0.99795	0.99541	0.99291	28.03
714	1.01294	1.01061	1.00829	1.00582	1.00335	1.00080	0.99824	0.99576	28.11
716	1.01594	1.01358	1.01118	1.00871	1.00624	1.00368	1.00112	0.99861	28.19
718	1.01894	1.01650	1.01406	1.01156	1.00906	1.00653	1.00400	1.00150	28.27
720	1.02194	1.01949	1.01694	1.01444	1.01194	1.00941	1.00688	1.00435	28.35
722	1.02492	1.02232	1.01982	1.01732	1.01482	1.01229	1.00976	1.00720	28.43
724	1.02771	1.02521	1.02271	1.02021	1.01771	1.01518	1.01265	1.01009	28.50
726	1.03059	1.02809	1.02559	1.02306	1.02053	1.01800	1.01547	1.01291	28.58
728	1.03347	1.03097	1.02847	1.02594	1.02341	1.02088	1.01835	1.01580	28.66
730	1.03635	1.03385	1.03135	1.02882	1.02629	1.02374	1.02118	1.01862	28.74
732	1.03924	1.03674	1.03424	1.03171	1.02918	1.02662	1.02406	1.02147	28.82
734	1.04218	1.03915	1.03712	1.03459	1.03206	1.02950	1.02694	1.02435	28.90
736	1.04506	1.04253	1.04000	1.03744	1.03488	1.03232	1.02976	1.02718	28.98
738	1.04794	1.04541	1.04288	1.04037	1.03776	1.03521	1.03265	1.03006	29.06
740	1.05082	1.04829	1.04576	1.04321	1.04065	1.03806	1.03547	1.03288	29.13
742	1.05371	1.05118	1.04865	1.04609	1.04353	1.04094	1.03835	1.03577	29.21
744	1.05659	1.05403	1.05147	1.04991	1.04635	1.04377	1.04118	1.03859	29.29
746	1.05947	1.05691	1.05435	1.05180	1.04924	1.04665	1.04406	1.04147	29.37
748	1.06235	1.05979	1.05724	1.05418	1.05212	1.04953	1.04694	1.04433	29.45
750	1.06524	1.06218	1.06012	1.05748	1.05494	1.05235	1.04976	1.04715	29.53
752	1.06818	1.06512	1.06306	1.06047	1.05788	1.05527	1.05265	1.05003	29.61
754	1.07106	1.06847	1.06588	1.06330	1.06071	1.05812	1.05553	1.05289	29.69
756	1.07394	1.07135	1.06876	1.06618	1.06359	1.06197	1.05935	1.05671	29.76
758	1.07682	1.07423	1.07165	1.06906	1.06647	1.06386	1.06124	1.05859	29.84
760	1.07971	1.07712	1.07453	1.07191	1.06929	1.06668	1.06406	1.06141	29.92
762	1.08259	1.08050	1.07741	1.07480	1.07218	1.06956	1.06694	1.06430	30.00
764	1.08547	1.08288	1.08029	1.07768	1.07506	1.07244	1.06982	1.06715	30.08
766	1.08841	1.08580	1.08318	1.08056	1.07794	1.07530	1.07265	1.06997	30.16
768	1.09129	1.08868	1.08606	1.08344	1.08082	1.07818	1.07553	1.07285	30.24
770	1.09418	1.09156	1.08894	1.08630	1.08365	1.08100	1.07835	1.07567	30.31
°F	59.0°	59.9°	60.8°	61.7°	62.6°	63.5°	64.4°	65.3°	

<sup>1</sup> Calculated from 1.976 = weight of 1 liter CO<sub>2</sub> at 0°C, 760 mm pressure and 41° latitude. Formula given by S. W. Parr, *J. Am. Chem. Soc.*, 31, 237 (1909).

**44.30** *Correction factors for the gasometric determination of carbon dioxide.*  
—Continued.

°C.	19.0°	19.5°	20.0°	20.5°	21.0°	21.5°	22.0°	22.5°	
<i>mm.</i>									<i>inches</i>
700	0.97385	0.97085	0.96835	0.96564	0.96294	0.96023	0.95753	0.95509	27.66
702	0.97618	0.97368	0.97118	0.96850	0.96582	0.96311	0.96041	0.95794	27.64
704	0.97906	0.97653	0.97400	0.97132	0.96865	0.96597	0.96329	0.96082	27.72
706	0.98188	0.97938	0.97688	0.97420	0.97153	0.96888	0.96624	0.96371	27.80
708	0.98476	0.98224	0.97971	0.97703	0.97435	0.97173	0.96912	0.96656	27.87
710	0.98759	0.98506	0.98253	0.97988	0.97724	0.97459	0.97195	0.96938	27.95
712	0.99041	0.98788	0.98535	0.98273	0.98012	0.97747	0.97483	0.97227	28.03
714	0.99329	0.99073	0.98818	0.98556	0.98294	0.98032	0.97771	0.97512	28.11
716	0.99612	0.99358	0.99106	0.98844	0.98582	0.98323	0.98065	0.97800	28.19
718	0.99900	0.99644	0.99388	0.99126	0.98865	0.98606	0.98348	0.98083	28.27
720	1.00182	0.99925	0.99671	0.99412	0.99153	0.98894	0.98636	0.98371	28.35
722	1.00465	1.00209	0.99953	0.99694	0.99435	0.99176	0.98918	0.98653	28.43
724	1.00753	1.00497	1.00241	0.99982	0.99724	0.99462	0.99200	0.98932	28.50
726	1.01035	1.00779	1.00524	1.00265	1.00006	1.99746	0.99483	0.99215	28.58
728	1.01324	1.01065	1.00806	1.00547	1.00288	1.00027	0.99765	0.99497	28.66
730	1.01606	1.01347	1.01088	1.00829	1.00571	1.00306	1.00041	1.99781	28.74
732	1.01888	1.01629	1.01371	1.01112	1.00853	1.00588	1.00324	1.00056	28.82
734	1.02176	1.01919	1.01659	1.01497	1.01135	1.00870	1.00606	1.00338	28.90
736	1.02459	1.02200	1.01941	1.01679	1.01418	1.01153	1.00888	1.00620	28.98
738	1.02747	1.02486	1.02224	1.01962	1.01700	1.01435	1.01171	1.00900	29.06
740	1.03029	1.02768	1.02506	1.02244	1.01982	1.01717	1.01453	1.01182	29.13
742	1.03318	1.03056	1.02794	1.02529	1.02265	1.02000	1.01735	1.01464	29.21
744	1.03600	1.03338	1.03076	1.02811	1.02547	1.02279	1.02012	1.01752	29.29
746	1.03888	1.03624	1.03359	1.03094	1.02829	1.02561	1.02294	1.02024	29.37
748	1.04171	1.03906	1.03641	1.03376	1.03112	1.02844	1.02576	1.02306	29.45
750	1.04453	1.04189	1.03924	1.03659	1.03394	1.03126	1.02859	1.02589	29.53
752	1.04741	1.04477	1.04212	1.03944	1.03676	1.03408	1.03141	1.02868	29.61
754	1.05024	1.04759	1.04494	1.04226	1.03959	1.03691	1.03424	1.03150	29.69
756	1.05306	1.05041	1.04776	1.04508	1.04241	1.03973	1.03706	1.03433	29.76
758	1.05594	1.05330	1.05065	1.04797	1.04529	1.04259	1.03988	1.03715	29.84
760	1.05876	1.05612	1.05347	1.05079	1.04812	1.04539	1.04265	1.03992	29.92
762	1.06165	1.05897	1.05629	1.05361	1.05094	1.04821	1.04547	1.04274	30.00
764	1.06447	1.06179	1.05912	1.05644	1.05376	1.05103	1.04829	1.04556	30.08
766	1.06729	1.06462	1.06194	1.05926	1.05659	1.05386	1.05112	1.04839	30.16
768	1.07018	1.06750	1.06482	1.06212	1.05941	1.05668	1.05394	1.05118	30.24
770	1.07300	1.07032	1.06765	1.06494	1.06224	1.05950	1.05676	1.05400	30.31
°F	66.2°	67.1°	68.0°	68.9°	69.8°	70.7°	71.6°	72.5°	

*Correction factors for the gasometric determination of carbon dioxide.* 44.30  
—Continued.

°C.	23.0°	23.5°	24.0°	24.5°	25.0°	25.5°	26.0°	26.5°	
mm.									inches
700	0.95265	0.95020	0.94776	0.94508	0.94241	0.93973	0.93706	0.93432	27.66
702	0.95547	0.95303	0.95059	0.94788	0.94518	0.94250	0.93982	0.93708	27.64
704	0.95835	0.95585	0.95335	0.95067	0.94800	0.94532	0.94265	0.93988	27.72
706	0.96118	0.95865	0.95612	0.95344	0.95076	0.94808	0.94541	0.94267	27.80
708	0.96400	0.96147	0.95894	0.95626	0.95359	0.95088	0.94818	0.94544	27.87
710	0.96682	0.96429	0.96176	0.95905	0.95635	0.95364	0.95094	0.94820	27.95
712	0.96971	0.96712	0.96453	0.96182	0.95912	0.95644	0.95376	0.95100	28.03
714	0.97253	0.96991	0.96729	0.96461	0.96194	0.95923	0.95653	0.95376	28.11
716	0.97535	0.97273	0.97012	0.96741	0.96471	0.96200	0.95929	0.95655	28.19
718	0.97819	0.97556	0.97294	0.97023	0.96753	0.96482	0.96212	0.95935	28.27
720	0.98106	0.97838	0.97571	0.97300	0.97029	0.96758	0.96488	0.96213	28.35
722	0.98388	0.98120	0.97853	0.97582	0.97312	0.97038	0.96765	0.96488	28.43
724	0.98665	0.98397	0.98129	0.97858	0.97588	0.97314	0.97041	0.96764	28.50
726	0.98947	0.98679	0.98412	0.98141	0.97871	0.97594	0.97318	0.97041	28.58
728	0.99229	0.98961	0.98694	0.98420	0.98147	0.97870	0.97594	0.97319	28.66
730	0.99512	0.99241	0.98971	0.98697	0.98424	0.98147	0.97871	0.97594	28.74
732	0.99788	0.99517	0.99247	0.98973	0.98700	0.98423	0.98147	0.97871	28.82
734	0.00071	0.99799	0.99529	0.99255	0.98982	0.98705	0.98429	0.98153	28.90
736	1.00353	1.00083	0.99812	0.99538	0.99265	0.98988	0.98706	0.98426	28.98
738	1.00629	1.00359	1.00088	0.99815	0.99541	0.99261	0.98982	0.98703	29.06
740	1.00912	1.00643	1.00371	1.00095	0.99818	0.99538	0.99259	0.98976	29.13
742	1.01194	1.00923	1.00653	1.00377	1.00100	0.99820	0.99541	0.99258	29.21
744	1.01471	1.01200	1.00929	1.00643	1.00376	1.00097	0.99818	0.99535	29.29
746	1.01753	1.01482	1.01212	1.00936	1.00659	1.00376	1.00094	0.99809	29.37
748	1.02035	1.01762	1.01488	1.01212	1.00935	1.00653	1.00371	1.00088	29.45
750	1.02318	1.02045	1.01771	1.01492	1.01212	1.00936	1.00659	1.00370	29.53
752	1.02594	1.02321	1.02047	1.01771	1.01494	1.01211	1.00929	1.00644	29.61
754	1.02876	1.02603	1.02329	1.02050	1.01771	1.01483	1.01206	1.00921	29.69
756	1.03159	1.02883	1.02606	1.02326	1.02047	1.01764	1.01482	1.01197	29.76
758	1.03441	1.03165	1.02888	1.02608	1.02329	1.02047	1.01765	1.01477	29.84
760	1.03718	1.03442	1.03165	1.02886	1.02606	1.02323	1.02041	1.01753	29.92
762	1.04000	1.03724	1.03447	1.03164	1.02882	1.02600	1.02318	1.02030	30.00
764	1.04282	1.04003	1.03723	1.03444	1.03165	1.02880	1.02594	1.02306	30.08
766	1.04565	1.04285	1.04005	1.03723	1.03441	1.03156	1.02871	1.02583	30.16
768	1.04841	1.04562	1.04282	1.04003	1.03724	1.03435	1.03147	1.02859	30.24
770	1.05123	1.04844	1.04564	1.04282	1.04000	1.03712	1.03424	1.03136	30.31
°F.	73.4°	74.3°	75.2°	76.1°	77.0°	77.9°	78.8°	79.7°	

**44.30** *Correction factors for the gasometric determination of carbon dioxide.*  
—Continued.

°C	27.0°	27.5°	28.0°	28.5°	29.0°	29.5°	30.0°	30.5°	
<i>mm.</i>									<i>inches</i>
700	0.93159	0.92885	0.92612	0.92332	0.92053	0.91773	0.91494	0.91203	27.56
702	0.93435	0.92161	0.92888	0.92608	0.92329	0.92047	0.91765	0.91476	27.64
704	0.93712	0.93438	0.93165	0.92882	0.92600	0.92320	0.92041	0.91750	27.72
706	0.93994	0.93717	0.93441	0.93158	0.92876	0.92594	0.92312	0.92024	27.80
708	0.94271	0.93994	0.93718	0.93435	0.93153	0.92870	0.92588	0.92297	27.87
710	0.94547	0.94267	0.93988	0.93706	0.93424	0.93141	0.92859	0.92567	27.95
712	0.94824	0.94544	0.94265	0.93982	0.93700	0.93414	0.93129	0.92841	28.03
714	0.95100	0.94820	0.94541	0.94258	0.93976	0.93691	0.93406	0.93115	28.11
716	0.95382	0.95100	0.94818	0.94535	0.94253	0.93969	0.93676	0.93388	28.19
718	0.95659	0.95376	0.95094	0.94809	0.94524	0.94238	0.93953	0.93662	28.27
720	0.95939	0.95655	0.95371	0.95085	0.94800	0.94512	0.94224	0.93932	28.35
722	0.96212	0.95929	0.95647	0.95361	0.95076	0.94788	0.94500	0.94209	28.43
724	0.96488	0.96206	0.95924	0.95638	0.95353	0.95062	0.94771	0.94479	28.50
726	0.96765	0.96482	0.96200	0.95912	0.95624	0.95332	0.95041	0.94750	28.58
728	0.97041	0.96758	0.96476	0.96188	0.95900	0.95609	0.95318	0.95026	28.66
730	0.97318	0.97036	0.96753	0.96464	0.96176	0.95885	0.95594	0.95300	28.74
732	0.97594	0.97309	0.97024	0.96735	0.96447	0.96156	0.95865	0.95578	28.82
734	0.97871	0.97585	0.97300	0.97012	0.96724	0.96429	0.96135	0.95844	28.90
736	0.98147	0.97861	0.97576	0.97288	0.97000	0.96706	0.96412	0.96118	28.98
738	0.98424	0.98138	0.97853	0.97564	0.97276	0.96982	0.96688	0.96394	29.06
740	0.98694	0.98409	0.98124	0.97835	0.97547	0.97253	0.96959	0.96665	29.13
742	0.98976	0.98691	0.98406	0.98115	0.97824	0.97529	0.97235	0.96941	29.21
744	0.99253	0.98967	0.98682	0.98391	0.98100	0.97806	0.97512	0.97215	29.29
746	0.99529	0.99241	0.98953	0.98662	0.98371	0.98076	0.97782	0.97485	29.37
748	0.99806	0.99517	0.99229	0.98938	0.98647	0.98353	0.98059	0.97762	29.45
750	1.00082	0.99796	0.99506	0.99215	0.98924	0.98626	0.98329	0.98032	29.53
752	1.00359	1.00071	0.99782	0.99491	0.99200	0.98903	0.98606	0.98306	29.61
754	1.00635	1.00342	1.00059	0.99738	0.99471	0.99173	0.98876	0.98579	29.69
756	1.00912	1.00624	1.00335	1.00041	0.99747	0.99450	0.99153	0.98853	29.76
758	1.01188	1.00900	1.00612	1.00318	1.00024	0.99724	0.99429	0.99129	29.84
760	1.01465	1.01174	1.00882	1.00588	1.00294	0.99995	0.99700	0.99400	29.92
762	1.01741	1.01450	1.01159	1.00865	1.00571	1.00274	0.99976	0.99673	30.00
764	1.02018	1.01727	1.01435	1.01141	1.00847	1.00547	1.00247	0.99948	30.08
766	1.02294	1.02003	1.01712	1.01418	1.01124	1.00824	1.00524	1.00221	30.16
768	1.02571	1.02280	1.01988	1.01691	1.01394	1.01094	1.00794	1.00491	30.24
770	1.02847	1.02556	1.02265	1.01968	1.01671	1.01371	1.01071	1.00768	30.31
°F	80.6°	81.5°	82.4°	83.3°	84.2°	85.1°	86.0°	86.9°	



*Correction factors for the gasometric determination of carbon dioxide.* 44.30  
—Concluded.

°C	31.0°	31.5°	32.0°	32.5°	33.0°	33.5°	34.0°	34.5°	35.0°	
mm.										inches
700	0.90912	0.90620	0.90329	0.90082	0.89735	0.89432	0.89129	0.88821	0.88512	27.56
702	0.91188	0.90894	0.90600	0.90303	0.90006	0.89703	0.89400	0.89091	0.88782	27.64
704	0.91459	0.91165	0.90871	0.90576	0.90282	0.89976	0.89671	0.89362	0.89053	27.72
706	0.91735	0.91441	0.91147	0.90847	0.90547	0.90241	0.89935	0.89627	0.89318	27.80
708	0.92006	0.91712	0.91418	0.91118	0.90818	0.90512	0.90206	0.89897	0.89588	27.87
710	0.92276	0.91982	0.91688	0.91388	0.91088	0.90782	0.90476	0.90168	0.89859	27.95
712	0.92553	0.92256	0.91959	0.91659	0.91359	0.91053	0.90747	0.90438	0.90129	28.03
714	0.92824	0.92529	0.92235	0.91932	0.91629	0.91323	0.91018	0.90706	0.90394	28.11
716	0.93100	0.92803	0.92506	0.92203	0.91900	0.91594	0.91288	0.90976	0.90665	28.19
718	0.93371	0.93078	0.92776	0.92474	0.92171	0.91865	0.91559	0.91247	0.90935	28.27
720	0.93641	0.93344	0.93047	0.92744	0.92441	0.92135	0.91829	0.91517	0.91206	28.35
722	0.93918	0.93618	0.93318	0.93015	0.92712	0.92412	0.92100	0.91785	0.91471	28.43
724	0.94188	0.93887	0.93586	0.93284	0.92982	0.92676	0.92371	0.92056	0.91741	28.50
726	0.94459	0.94159	0.93859	0.93556	0.93253	0.92944	0.92635	0.92323	0.92012	28.58
728	0.94735	0.94435	0.94135	0.93830	0.93524	0.93215	0.92906	0.92591	0.92276	28.66
730	0.95006	0.94706	0.94406	0.94103	0.93800	0.93488	0.93176	0.92861	0.92547	28.74
732	0.95282	0.94979	0.94676	0.94373	0.94071	0.93759	0.93447	0.93132	0.92818	28.82
734	0.95553	0.95250	0.94947	0.94644	0.94341	0.94034	0.93718	0.93403	0.93088	28.90
736	0.95824	0.95521	0.95218	0.94915	0.94612	0.94300	0.93988	0.93670	0.93353	28.98
738	0.96100	0.95797	0.95494	0.95188	0.94882	0.94570	0.94259	0.93941	0.93624	29.06
740	0.96371	0.96068	0.95765	0.95459	0.95153	0.94841	0.94529	0.94211	0.93894	29.13
742	0.96647	0.96341	0.96035	0.95730	0.95424	0.95112	0.94800	0.94482	0.94165	29.21
744	0.96918	0.96615	0.96312	0.96003	0.95694	0.95382	0.95071	0.94750	0.94429	29.29
746	0.97188	0.96885	0.96582	0.96273	0.95965	0.95653	0.95341	0.95020	0.94700	29.37
748	0.97465	0.97159	0.96853	0.96544	0.96235	0.95925	0.95606	0.95288	0.94971	29.45
750	0.97735	0.97429	0.97124	0.96815	0.96506	0.96191	0.95876	0.95558	0.95241	29.53
752	0.98006	0.97703	0.97400	0.97088	0.96776	0.96461	0.96147	0.95826	0.95506	29.61
754	0.98282	0.97976	0.97671	0.97359	0.97047	0.96732	0.96418	0.96097	0.95776	29.69
756	0.98553	0.98247	0.97941	0.97629	0.97318	0.97003	0.96688	0.96367	0.96047	29.76
958	0.98829	0.98521	0.98212	0.97900	0.97588	0.97273	0.96959	0.96638	0.96318	29.84
760	0.99100	0.98794	0.98488	0.98176	0.97865	0.97547	0.97229	0.96908	0.96588	29.92
762	0.99371	0.99065	0.98759	0.98443	0.98135	0.97817	0.97500	0.97176	0.96853	30.00
764	0.99647	0.99338	0.99029	0.98717	0.98408	0.98098	0.97771	0.97447	0.97124	30.08
766	0.99918	0.99609	0.99300	0.98988	0.98676	0.98356	0.98033	0.97714	0.97394	30.16
768	1.00188	0.99880	0.99571	0.99259	0.98947	0.98629	0.98312	0.97986	0.97659	30.24
770	1.00465	1.00156	0.99847	0.99532	0.99218	0.98897	0.98576	0.98252	0.97929	30.31
°F	87.8°	88.7°	89.6°	90.5°	91.4°	92.3°	93.2°	94.1°	95.0°	

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*Progressive accumulation of radium emanation<sup>1</sup>*

$$I_t = I_0(1 - e^{-\lambda t}); \lambda = 0.1801 \text{ days}^{-1}; I_0 = 1$$

HOURS	0 DAYS	$\Delta$	1 DAY	$\Delta$	2 DAYS	$\Delta$	3 DAYS	$\Delta$	4 DAYS	$\Delta$	5 DAYS	$\Delta$	6 DAYS	$\Delta$	7 DAYS	$\Delta$
0	.0000	75	.1648	68	.3025	52	.4175	44	.5136	36	.5937	31	.6607	25	.7166	22
1	.0075	74	.1711	62	.3077	52	.4219	43	.5172	36	.5963	30	.6632	26	.7188	20
2	.0149	74	.1773	61	.3129	52	.4262	43	.5208	36	.5998	30	.6658	24	.7208	21
3	.0223	73	.1834	62	.3181	51	.4305	42	.5244	35	.6028	29	.6682	25	.7229	21
4	.0296	72	.1896	60	.3232	50	.4347	42	.5279	35	.6057	30	.6707	25	.7250	21
5	.0368	72	.1956	60	.3282	50	.4390	42	.5314	36	.6087	29	.6732	24	.7271	20
6	.0440	72	.2016	60	.3332	50	.4432	41	.5350	34	.6116	29	.6756	25	.7291	21
7	.0512	71	.2076	59	.3382	50	.4473	41	.5384	35	.6145	29	.6781	24	.7311	20
8	.0583	70	.2135	59	.3432	49	.4514	42	.5419	34	.6174	29	.6805	24	.7332	19
9	.0653	70	.2194	58	.3481	49	.4556	40	.5453	34	.6203	28	.6829	23	.7351	20
10	.0723	69	.2252	58	.3530	48	.4596	41	.5487	34	.6231	28	.6852	24	.7371	20
11	.0792	69	.2310	58	.3578	48	.4637	40	.5521	33	.6259	28	.6876	23	.7391	20
12	.0861	69	.2368	57	.3626	48	.4677	40	.5554	34	.6287	28	.6899	23	.7410	20
13	.0930	68	.2425	57	.3674	47	.4717	39	.5588	33	.6315	27	.6922	23	.7430	19
14	.0998	67	.2482	56	.3721	47	.4756	39	.5621	32	.6342	28	.6945	23	.7449	19
15	.1065	67	.2538	56	.3768	46	.4795	39	.5653	33	.6370	27	.6968	23	.7468	19
16	.1132	66	.2594	55	.3814	47	.4834	39	.5686	32	.6397	27	.6991	23	.7487	19
17	.1198	66	.2649	55	.3861	46	.4873	38	.5718	32	.6424	27	.7014	22	.7506	18
18	.1264	65	.2704	54	.3907	45	.4911	38	.5750	32	.6451	26	.7036	22	.7524	19
19	.1329	65	.2758	55	.3952	46	.4949	38	.5782	31	.6477	27	.7058	22	.7543	18
20	.1394	64	.2813	53	.3998	44	.4987	37	.5813	32	.6504	26	.7080	22	.7561	19
21	.1458	64	.2866	54	.4042	45	.5024	38	.5845	31	.6530	26	.7102	22	.7580	18
22	.1522	64	.2920	53	.4087	44	.5062	37	.5876	31	.6556	25	.7124	21	.7598	18
23	.1586	62	.2973	52	.4131	44	.5099	37	.5907	30	.6581	26	.7145	21	.7616	18

HOURS	8 DAYS	$\Delta$	9 DAYS	$\Delta$	10 DAYS	$\Delta$	11 DAYS	$\Delta$	12 DAYS	$\Delta$	13 DAYS	$\Delta$	14 DAYS	$\Delta$	15 DAYS	$\Delta$
0	.7634	17	.8024	14	.8349	13	.8622	10	.8849	8	.9038	8	.9197	6	.9329	5
1	.7651	18	.8038	15	.8362	12	.8632	10	.8857	9	.9046	7	.9203	6	.9334	5
2	.7669	17	.8053	15	.8374	12	.8642	10	.8866	8	.9053	7	.9209	6	.9339	5
3	.7686	18	.8068	14	.8386	12	.8652	10	.8874	9	.9060	7	.9215	6	.9344	5
4	.7704	17	.8082	14	.8398	12	.8662	10	.8883	8	.9067	7	.9221	6	.9349	5
5	.7721	17	.8096	15	.8410	12	.8672	10	.8891	8	.9074	7	.9227	5	.9354	5
6	.7738	17	.8111	14	.8422	12	.8682	10	.8899	9	.9081	7	.9232	6	.9359	5
7	.7755	16	.8125	15	.8434	12	.8692	10	.8908	8	.9088	7	.9238	6	.9364	4
8	.7771	17	.8139	14	.8446	11	.8702	10	.8916	8	.9095	6	.9244	6	.9368	5
9	.7788	17	.8153	13	.8457	12	.8712	9	.8924	8	.9101	7	.9250	5	.9373	5
10	.7805	16	.8166	14	.8469	11	.8721	10	.8932	8	.9108	7	.9255	6	.9378	4
11	.7821	16	.8180	14	.8480	12	.8731	9	.8940	8	.9115	6	.9261	5	.9382	5
12	.7837	17	.8194	13	.8492	11	.8740	10	.8948	8	.9121	7	.9266	6	.9387	5
13	.7854	16	.8207	14	.8503	11	.8750	9	.8956	8	.9128	6	.9272	5	.9392	4
14	.7870	16	.8221	13	.8514	11	.8759	9	.8964	7	.9134	7	.9277	5	.9396	5
15	.7886	15	.8234	13	.8525	11	.8768	9	.8971	8	.9141	6	.9282	6	.9401	4
16	.7901	16	.8247	13	.8536	11	.8777	10	.8979	8	.9147	7	.9288	5	.9405	5
17	.7917	15	.8260	13	.8547	11	.8787	9	.8987	7	.9154	6	.9293	5	.9410	4
18	.7932	16	.8273	13	.8558	11	.8796	9	.8994	8	.9160	6	.9298	6	.9414	4
19	.7948	15	.8286	13	.8569	11	.8805	9	.9002	7	.9166	7	.9304	5	.9418	5
20	.7963	16	.8299	13	.8580	10	.8814	8	.9009	8	.9173	6	.9309	5	.9423	4
21	.7979	15	.8312	12	.8590	11	.8822	9	.9017	7	.9179	6	.9314	5	.9427	5
22	.7994	15	.8324	13	.8601	10	.8831	9	.9024	7	.9185	6	.9319	5	.9432	4
23	.8009	15	.8337	12	.8611	11	.8840	9	.9031	7	.9191	6	.9324	5	.9436	4

<sup>1</sup> Univ. of Missouri Bull. 14, No. 26, 85 (1923).

*Progressive accumulation of radium emanation—Concluded.*

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HOURS	16 DAYS	$\Delta$	17 DAYS	$\Delta$	18 DAYS	$\Delta$	19 DAYS	$\Delta$	20 DAYS	$\Delta$	21 DAYS	$\sigma$	22 DAYS	$\Delta$	23 DAYS	$\Delta$
0	.9440	9	.9533	7	.9610	6	.9674	5	.9728	4	.9773	3	.9810	3	.9842	2
2	.9449	8	.9540	6	.9616	5	.9679	5	.9732	4	.9776	4	.9813	3	.9844	2
4	.9457	8	.9546	7	.9621	6	.9684	5	.9736	4	.9780	3	.9816	3	.9846	3
6	.9465	8	.9553	7	.9627	6	.9689	4	.9740	4	.9783	3	.9819	3	.9849	2
8	.9473	8	.9560	6	.9633	5	.9693	5	.9744	4	.9788	3	.9822	2	.9851	2
10	.9481	8	.9566	7	.9638	5	.9698	4	.9748	4	.9789	3	.9824	3	.9853	2
12	.9489	7	.9573	6	.9643	6	.9702	5	.9752	3	.9792	4	.9827	2	.9855	2
14	.9496	8	.9579	7	.9649	5	.9707	4	.9755	4	.9796	3	.9829	3	.9857	2
16	.9504	7	.9586	6	.9654	5	.9711	4	.9759	3	.9799	3	.9832	2	.9859	2
18	.9511	7	.9592	6	.9659	5	.9715	5	.9762	4	.9802	3	.9834	3	.9861	2
20	.9518	8	.9598	6	.9664	5	.9720	4	.9766	3	.9805	3	.9837	2	.9863	2
22	.9526	7	.9604	6	.9669	5	.9724	4	.9769	4	.9808	2	.9839	3	.9865	2

HOURS	24 DAYS	$\Delta$	25 DAYS	$\Delta$	26 DAYS	$\Delta$	27 DAYS	$\Delta$	28 DAYS	$\Delta$	29 DAYS	$\Delta$	30 DAYS	$\Delta$	31 DAYS	$\Delta$
0	.9867	4	.9889	4	.9908	2	.9923	2	.9936	1	.9947	1	.9955	2	.9963	1
4	.9871	4	.9893	3	.9910	3	.9925	2	.9937	2	.9948	2	.9957	1	.9964	1
8	.9875	4	.9896	3	.9913	3	.9927	2	.9939	2	.9950	1	.9958	1	.9965	1
12	.9879	3	.9899	3	.9916	2	.9929	3	.9941	2	.9951	2	.9959	1	.9966	1
16	.9882	4	.9902	3	.9918	2	.9932	2	.9943	2	.9953	1	.9960	2	.9967	1
20	.9886	3	.9905	3	.9920	3	.9934	2	.9945	2	.9954	1	.9962	1	.9968	1

## APPENDIX

### DEFINITIONS OF TERMS AND INTERPRETATIONS OF RESULTS ON FERTILIZERS AND LIMING MATERIALS

#### DEFINITIONS

##### ACID-FORMING AND NON-ACID-FORMING FERTILIZERS

An *acid-forming fertilizer* is one that is capable of increasing the residual acidity of soil (adopted 1936).

A *non-acid-forming fertilizer* is one that is not capable of increasing the residual acidity of the soil (adopted 1936).

##### ACIDULATED FISH TANKAGE, ACIDULATED FISH SCRAP

*Acidulated fish tankage, acidulated fish scrap*, is the rendered product derived from fish and treated with sulfuric acid (adopted 1930).

##### ACTIVATED SEWAGE PRODUCTS

*Activated sewage products* are those made from sewage freed from grit and coarse solids and aerated after being inoculated with microorganisms. The resulting flocculated organic matter is withdrawn from the tanks, filtered with or without the aid of coagulants, dried, ground, and screened (adopted 1930).

##### AGRICULTURAL LIMING MATERIAL

*Agricultural liming material* is material whose calcium and magnesium content is capable of neutralizing soil acidity (adopted 1935).

##### AIR-SLAKED LIME

*Air-slaked lime* is a product composed of varying proportions of the oxide, hydroxide, and carbonate of calcium, or of calcium and magnesium, and derived from exposure of quicklime (adopted 1936).

##### AMMONIATED SUPERPHOSPHATE

*Ammoniated superphosphate* is the product obtained when superphosphate is treated with ammonia or with a solution containing free ammonia and other forms of nitrogen dissolved therein (adopted 1940).

##### ANALYSIS

The word *analysis*, as applied to fertilizers, shall designate the percentage composition of the product expressed in those terms that the law requires and permits (adopted 1938).

##### ASHES FROM LEACHED WOOD

*Ashes from leached wood* are unleached ashes resulting from burning wood that has been exposed to or digested in water or other liquid solvent, as in the extraction of dyes, so that a part of the plant food has been dissolved and removed (adopted 1926).

##### AVAILABLE PHOSPHORIC ACID

*Available phosphoric acid* is the sum of the water-soluble and the citrate-soluble phosphoric acid (adopted 1931).

**"BASIC" LIME PHOSPHATE**

*"Basic" lime phosphate (lime-based superphosphate)* is a superphosphate to which liming materials have been added in a quantity at least six per cent (6%) calcium carbonate equivalents in excess of the quantity required to convert all water-soluble phosphate to the citrate-soluble form (adopted 1934).

**BASIC PHOSPHATE SLAG**

*Basic phosphate slag* is a by-product in the manufacture of steel from phosphatic iron ores. The product shall be finely ground and shall contain no admixture of materials other than what results in the original process of manufacture. It shall contain not less than twelve per cent (12%) of total phosphoric acid ( $P_2O_5$ ), not less than eighty per cent (80%) of which shall be soluble in two per cent (2%) citric acid solution according to the Wagner method of analysis, 2.19 or 2.20. Any phosphate slag not conforming to this definition shall be designated *low grade* (adopted 1925).

**BAT GUANO**

*Bat guano* is partially decomposed bat manure (adopted 1938).

**BAT MANURE**

*Bat manure* is the dry excrement from bats (adopted 1938).

**BRAND AND BRAND NAME**

A *brand* is a term, design, or trademark used in connection with one or several grades of fertilizers (adopted 1926).

A *brand name* is a specific designation applied to an individual fertilizer (adopted 1926).

**CALCIUM NITRATE**

*Calcium nitrate* (nitrate of lime) is a commercial product consisting chiefly of calcium nitrate, and it shall contain not less than fifteen per cent (15%) of nitrogen (adopted 1940).

**CITRATE-SOLUBLE ("REVERTED") PHOSPHORIC ACID**

*Citrate-soluble ("reverted") phosphoric acid* is that part of the total phosphoric acid in a fertilizer that is insoluble in water but soluble in a solution of citrate of ammonia according to the method adopted by the A.O.A.C. (adopted 1932).

**CRUDE, INERT, OR SLOW-ACTING NITROGENOUS MATERIALS**

*Crude, inert, or slow-acting nitrogenous materials* are unprocessed organic substances relatively high in nitrogen but having a very low value as plant food and showing a low activity by both the alkaline and neutral permanganate methods (below 50% and 80%, respectively), (adopted 1929).

**CYANAMID**

*Cyanamid* is a commercial product composed chiefly of calcium cyanamide ( $CaCN_2$ ), and it shall contain not less than twenty-one per cent (21%) of nitrogen (adopted 1935).

**DICALCIUM PHOSPHATE**

*Dicalcium phosphate* is a manufactured product consisting chiefly of a dicalcic salt of phosphoric acid (adopted 1931).

**DISSOLVED BONE**

*Dissolved bone* is ground bone or bone meal that has been treated with sulfuric acid (adopted 1926).

**DOLOMITE**

*Dolomite* is a mineral composed chiefly of carbonates of magnesium and calcium in substantially unimolal (1-1.19) proportions (adopted 1938).

**DRIED BLOOD**

*Dried blood* is the collected blood of slaughtered animals, dried and ground and containing not less than twelve per cent (12%) of nitrogen in organic forms (adopted 1928).

**DRIED, PULVERIZED, OR SHREDDED MANURES**

*Dried, pulverized, or shredded manures* are what the name indicates, and not mixtures of manures and other materials (adopted 1925).

**FERTILIZER GRADE**

*Fertilizer grade* shall represent the minimum guaranty of its plant food expressed in terms of *nitrogen (not ammonia)*, *available phosphoric acid*, and *water-soluble potash* (adopted 1928).

**FISH TANKAGE, FISH SCRAP, DRY GROUND FISH, FISH MEAL FERTILIZER GRADE**

*Fish tankage, fish scrap, dry ground fish, fish meal fertilizer grade*, is the dried ground product derived from rendered or unrendered fish (adopted 1929).

**GARBAGE TANKAGE**

*Garbage tankage* is the rendered, dried, and ground product derived from waste household food materials (adopted 1929).

**PULVERIZED LIMESTONE (FINE-GROUND LIMESTONE)**

*Pulverized limestone (fine-ground limestone)* is the product obtained by grinding either calcitic or dolomitic limestone so that all the material will pass a 20-mesh sieve and at least seventy-five per cent (75%) will pass a 100-mesh sieve (adopted 1936).

**GROUND LIMESTONE (COARSE-GROUND LIMESTONE)**

*Ground limestone (coarse-ground limestone)* is the product obtained by grinding either calcitic or dolomitic limestone so that all the material will pass a 10-mesh sieve, and at least fifty per cent (50%) will pass a 100-mesh sieve (adopted 1936).

**GROUND SHELLS**

*Ground shells* is the product obtained by grinding the shells of mollusks so that not less than fifty per cent (50%) shall pass a 100-mesh sieve. The product shall also carry the name of the mollusk from which said product is made (adopted 1936).

**GROUND SHELL MARL**

*Ground shell marl* is the product obtained by grinding natural deposits of shell marl so that at least seventy-five per cent (75%) shall pass a 100-mesh sieve (adopted 1936).

**GROUND RAW BONE**

*Ground raw bone* is dried ground animal bones that have not been previously steamed under pressure (adopted 1929).

**GROUND STEAMED BONE**

*Ground steamed bone* is ground animal bones that have been previously steamed under pressure (adopted 1929).

**GYPSUM, LAND PLASTER, OR CRUDE CALCIUM SULFATE**

*Gypsum, land plaster, or crude calcium sulfate* is a product consisting chiefly of calcium sulfate. It may contain twenty per cent (20%) of combined water (it does not neutralize acid soils) (adopted 1931).

**HIGH CALCIC PRODUCTS**

*High calcic products* are materials of which 90% or more of the total calcium and magnesium oxide content consists of calcium oxide (adopted 1935).

**HIGH MAGNESIC PRODUCTS**

*High magnesian products* are materials in which more than 10 per cent of the total calcium and magnesium oxide consists of magnesium oxide (adopted 1935).

**HOOF AND HORN MEAL**

*Hoof and horn meal* is processed dried, ground hoofs and horns (adopted 1929).

**HYDRATED OR SLAKED LIME**

*Hydrated or slaked lime* is a dry product consisting chiefly of the hydroxide of calcium and oxide-hydroxide of magnesium (adopted 1935).

**KAINIT**

*Kainit* is a potash salt containing potassium and sodium chlorides and sometimes sulfate of magnesia with not less than twelve per cent (12%) of potash ( $K_2O$ ) (adopted 1928).

**LEACHED WOOD ASHES**

*Leached wood ashes* are ashes from burned unleached wood with part of their plant food removed by artificial means or by exposure to rains, snows, or other solvent (adopted 1928).

**LIME**

The word *lime* when applied to liming materials means either calcium oxide or calcium and magnesium oxides (adopted 1934).

**MAGNESIA**

*Magnesia* (magnesium oxide) is a product consisting chiefly of the oxide of magnesium. Its grade shall be stipulated. Example: Magnesia—75 per cent  $MgO$  (adopted 1944).

**MANGANESE**

*Manganese.* The water-soluble (or available) manganese in fertilizers shall be expressed as manganese (Mn) (adopted 1935).

**MANGANESE SULFATE**

*Manganese sulfate.* The term manganese sulfate, when applied to an ingredient of a mixed fertilizer, shall designate anhydrous manganous sulfate ( $\text{MnSO}_4$ ) (adopted 1935).

**MANURE SALTS**

*Manure salts* are potash salts containing high percentages of chloride and from twenty per cent (20%) to thirty per cent (30%) of potash ( $\text{K}_2\text{O}$ ). The term *double manure salts* should be discontinued (adopted 1925).

**MONOAMMONIUM PHOSPHATE (FERTILIZER GRADE)**

*Monoammonium phosphate (fertilizer grade)* is a commercial salt made by combining phosphoric acid with ammonia. It shall contain not less than ten per cent (10%) of nitrogen and not less than forty-six per cent (46%) of available phosphoric acid (adopted 1934).

**MURIATE OF POTASH (COMMERCIAL POTASSIUM CHLORIDE)**

*Muriate of potash* (commercial potassium chloride) is a potash salt containing not less than forty-eight per cent (48%) of potash ( $\text{K}_2\text{O}$ ), chiefly as chlorides (adopted 1929).

**NITRATE OF AMMONIA (AMMONIUM NITRATE)**

*Nitrate of ammonia* (ammonium nitrate) is a product composed chiefly of nitrate of ammonium. Its nitrogen content shall be stipulated. Example: Ammonium nitrate—30 per cent N (adopted 1944).

**NITRATE OF POTASH (COMMERCIAL POTASSIUM NITRATE)**

*Nitrate of potash* (commercial potassium nitrate) is a salt containing not less than twelve per cent (12%) of nitrogen and forty-four per cent (44%) of potash ( $\text{K}_2\text{O}$ ) (adopted 1927).

**NITRATE OF SODA (COMMERCIAL SODIUM NITRATE)**

*Nitrate of soda* (commercial sodium nitrate) is commercial sodium nitrate containing not less than fifteen per cent (15%) of nitrogen, chiefly as sodium nitrate (adopted 1928).

**NITRATE OF SODA AND POTASH**

*Nitrate of soda and potash* is a commercial product containing nitrates of sodium and potassium, and it shall contain not less than fourteen per cent (14%) of nitrogen (N) and fourteen per cent (14%) of potash ( $\text{K}_2\text{O}$ ) (adopted 1943).

**PEAT**

*Peat* is partly decayed vegetable matter of natural occurrence. It is composed chiefly of organic matter that contains some nitrogen of low activity (adopted 1931).

**CHARRED PEAT**

*Charred peat* is peat artificially dried at a temperature that causes partial decomposition (adopted 1931).

**PHOSPHATE ROCK**

*Phosphate rock* is a natural rock containing one or more calcium phosphate minerals of sufficient purity and quantity to permit its use, either directly or after concentration, in the manufacture of commercial products (adopted 1933).



**PHOSPHORIC ACID**

The term *phosphoric acid* designates phosphorus pentoxide ( $P_2O_5$ ) (adopted 1934).

**POTASH**

The term *potash* designates potassium oxide ( $K_2O$ ) (adopted 1934).

**PRECIPITATED BONE PHOSPHATE**

*Precipitated bone phosphate* is a by-product from the manufacture of glue from bones and is obtained by neutralizing the hydrochloric acid solution of processed bone with calcium hydroxide. The phosphoric acid is chiefly present as dicalcium phosphate (adopted 1933).

**PRECIPITATED PHOSPHATE**

*Precipitated phosphate* is a product consisting mainly of dicalcium phosphate obtained by neutralizing with calcium hydroxide the acid solution of either phosphate rock or processed bone (adopted 1933).

**PRIMARY FERTILIZER COMPONENTS**

*Primary fertilizer components* are those at present generally recognized by law as necessary to be guaranteed in fertilizers, namely: nitrogen, phosphoric acid ( $P_2O_5$ ), and potash ( $K_2O$ ) (adopted 1938).

**SECONDARY FERTILIZER COMPONENTS**

*Secondary fertilizer components* are those other than the "primary fertilizer components" that are essential to the proper growth of plants and that may be needed by some soils. Some of these components are calcium, magnesium, sulfur, manganese, copper, zinc, and boron (adopted 1938).

**PROCESS TANKAGES**

*Process tankages* are products made under steam pressure from crude inert nitrogenous materials, with or without the use of acids, for the purpose of increasing the activity of the nitrogen. These products shall be called "Process Tankages" with or without further qualification. The water-insoluble nitrogen in these products shall test at least fifty per cent (50%) active by the alkaline, or eighty per cent (80%) by the neutral permanganate method (adopted 1931).

**PRODUCTS SECURED BY HEATING CALCIUM PHOSPHATE WITH ALKALI SALTS CONTAINING POTASH**

*Products secured by heating calcium phosphate with alkali salts containing potash* are non-acid phosphates with potash. They are *not* potassium phosphate (adopted 1928).

**QUICK LIME, BURNED LIME, CAUSTIC LIME, LUMP LIME, UNSLAKED LIME**

*Quick lime, burned lime, caustic lime, lump lime, unslaked lime.* These designations shall apply to calcined materials, the major part of which is calcium oxide, in natural association with a lesser amount of magnesium oxide, and which is capable of slaking with water (adopted 1935).

**SHEEP MANURE—WOOL WASTE**

*Sheep manure—wool waste* is the by-product from wool-carding establishments consisting chiefly of sheep manure, seeds, and wool fiber (adopted 1931).

**SOFT PHOSPHATE WITH COLLOIDAL CLAY**

*Soft phosphate with colloidal clay* is a very finely divided low-analysis by-product from mining Florida rock phosphate by a hydraulic process in which the colloidal materials settle at points in artificial ponds and basins farthest from the washer, and are later removed after the natural evaporation of the water (adopted 1933).

**SULFATE OF AMMONIA (COMMERCIAL AMMONIUM SULFATE)**

*Sulfate of ammonia (commercial ammonium sulfate)* is a commercial product composed chiefly of ammonium sulfate. It shall contain not less than twenty and five-tenths per cent (20.5%) of nitrogen (adopted 1931).

**SULFATE OF POTASH-MAGNESIA**

*Sulfate of potash-magnesia* is a potash salt containing not less than twenty-five per cent (25%) of potash ( $K_2O$ ), nor less than twenty-five per cent (25%) of sulfate of magnesia, and not more than two and one-half per cent (2.5%) of chlorine (adopted 1925).

**SULFATE OF POTASH (COMMERCIAL POTASSIUM SULFATE)**

*Sulfate of potash (commercial potassium sulfate)* is a potash salt containing not less than forty-eight per cent (48%) of potash ( $K_2O$ ) chiefly as sulfate, and not more than two and one-half per cent (2.5%) of chlorine (adopted 1929).

**SUPERPHOSPHATE**

*Superphosphate* is a product obtained by mixing rock phosphate with either sulfuric acid or phosphoric acid or with both acids. (The grade that shows the available phosphoric acid shall be used as a prefix to the name. Example: 20 per cent superphosphate) (adopted 1940).

**TANKAGE**

*Tankage* (without qualification) is the rendered, dried, and ground by-product, largely meat and bone from animals (slaughtered or that have died otherwise) (adopted 1929).

**UNIT OF PLANT FOOD**

*A unit of plant food* is twenty (20) pounds, or one per cent (1%) of a ton (adopted 1926).

**UNLEACHED WOOD ASHES**

*Unleached wood ashes* are ashes from burned unleached wood that have had no part of their plant food removed and that contain four per cent (4%) or more of water-soluble potash ( $K_2O$ ) (adopted 1928).

**WASTE LIME, BY-PRODUCT LIME**

*Waste lime, by-product lime*, is any industrial waste or by-product containing calcium or calcium and magnesium in forms that will neutralize acids. It may be designated by prefixing the name of the industry or process by which it is produced, i.e., gas-house lime, tanners' lime, acetylene lime-waste, lime-kiln ashes, calcium silicate, etc. (adopted 1931).

## INTERPRETATIONS

## ACTIVITY OF WATER-INSOLUBLE NITROGEN IN MIXED FERTILIZERS

*Activity of water-insoluble nitrogen in mixed fertilizers.*—The alkaline and neutral permanganate methods distinguish between the better and the poorer sources of water-insoluble nitrogen, and do not show the percentage availability of the materials. The available nitrogen of any product can be measured only after carefully conducted vegetation experiments.

(a) The methods shall be used on mixed fertilizers containing water-insoluble nitrogen amounting to three-tenths of one per cent (0.3%) or more of the weight of the material. If a total nitrogen exceeds the minimum guaranty and is accompanied by a low activity of the insoluble nitrogen, the over-run shall be taken into consideration in determining the classification of the water-insoluble nitrogen.

(b) The water-insoluble nitrogen in mixed fertilizers showing an activity below fifty per cent (50%) by the alkaline method and also below eighty per cent (80%) by the neutral method shall be classed as inferior. This necessitates the use of both methods, also the provision as to over-run in (a), before classifying as inferior (adopted 1927).

## AMOUNT OF CHLORINE PERMISSIBLE IN FERTILIZERS IN WHICH THE POTASH IS CLAIMED AS SULFATE

*Amount of chlorine permissible in fertilizers in which the potash is claimed as sulfate.*—The chlorine in mixed fertilizers in which the potash is claimed as sulfate shall not exceed one-half of one per cent (0.5%) more than what is called for in the minimum potash content based on the definition of sulfate of potash as formulated by the Committee. Calculate as follows: 0.05 times the percentage of potash found plus 0.5 (adopted 1928).

## BRAND NAME TO INCLUDE GRADE OF FERTILIZER

*The grade of a fertilizer* should be included with its brand name, and so used by the manufacturer on sacks and in printed literature and by the control official in his reports and publications (adopted 1927).

## CYANAMIDE AND UREA NITROGEN

*The nitrogen in cyanamide and urea* is synthetic, non-protein organic nitrogen (adopted 1931).

## FERTILIZER FORMULA

*A fertilizer formula* shall express the quantity and grade of the crude stock materials used in making a fertilizer mixture. For example: 800 pounds of 16% superphosphate, 800 pounds of tankage (7.40 nitrogen and 9.15 total phosphoric acid), and 400 pounds of sulfate of potash-magnesia (twenty-six per cent (26%) potash) (adopted 1926).

## FINELY GROUND AS APPLIED TO BASIC PHOSPHATE SLAG

*Finely ground* in the definition of basic phosphate slag shall refer to actual size of particles as determined by the use of standard sieves, as follows: seventy per cent (70%) or more shall pass a 100-, and ninety per cent (90%) or more shall pass a 50-mesh sieve (adopted 1927).

**GUARANTEEING IN TERMS OF ELEMENTS**

All fertilizer components, with the exceptions of potash ( $K_2O$ ) and phosphoric acid ( $P_2O_5$ ) if guaranteed, shall be stated in terms of the elements (adopted 1944, first action).

**LABELLING OF NITRATES**

It is recommended that bags of fertilizer nitrates shall carry the warning "*injurious to livestock*" (adopted 1944).

**"LIME" AS APPLIED TO FERTILIZERS**

The term "*lime*" shall not be used in the registration, labelling, or guaranteeing of fertilizers or fertilizing materials unless the lime is in a form or forms to neutralize soil acidity (adopted 1935).

**NET WEIGHTS**

The weights appearing on packages of fertilizer, agricultural lime, and liming materials shall always mean *net weights* (adopted 1932).

**ORDER OF TERMS**

The *order of terms* in mixed fertilizers shall be nitrogen first, phosphoric acid second, and potash third (adopted 1930).

**NAME OF A FERTILIZER MATERIAL USED AS THE BRAND NAME OR PART OF THE BRAND NAME OF A MIXED FERTILIZER**

When the name of a fertilizer material is used as a part of the brand name of a mixed fertilizer, as for example, blood, bone, or fish, the nitrogen or phosphoric acid shall be derived from or supplied entirely by the material named. When the name of a fertilizer material is used as a brand or as part of a brand and the nitrogen or phosphoric acid is not supplied by the material named, the word "brand" shall follow the name of the materials. Example: "Fish Brand Fertilizer" (adopted 1930).

**STATEMENT OF GUARANTIES**

The *statement of guaranties* of mixed fertilizers shall be given in whole numbers (adopted 1930).

**UNIFORMITY IN USE OF TERMS "PHOSPHORIC ACID" AND "POTASH"**

As the terms *phosphoric acid* and *potash* are used universally in guaranteeing and in reporting the analyses of fertilizers, it is recommended that the same terms also be used in reporting and discussing the results of analyses of related materials (adopted 1934).



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